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Effect of zinc on immune function in young swine and on models of susceptibility to Serpulina hyodysenteriae infection

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Iowa State University, 1994

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Effect of zinc on immune function in young swine and on models of susceptibility to *Serpulina hyodysenteriae* infection

by

Vickie Lynn Jewell Hall

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GENERAL INTRODUCTION

Zinc is widely accepted as an important nutritional factor for maintaining immune functions. Zinc deficiency influences many factors important to host defense mechanisms including T cell function, antibody production, thymic hormone levels, and cytokine activity. The classic sign of zinc deficiency in pigs is parakeratosis, but impaired immune function is also an implied effect of zinc deficiency in pigs. Observations which support the impairment of the immune system in zinc deficient pigs include reduced thymic weight, elevated numbers of leukocytes (including immature cells), and increased mortality rate in the face of bacterial challenge.

Although mice have been used extensively as a model for evaluating the effects of zinc deficiency on immune functions, little is known about the effects of zinc on immune functions in the pig. These studies were conducted to evaluate two hypotheses: 1) that zinc deficiency in swine will result in impaired immune functions similar to those described for other species, and 2) that the chelate, zinc methionine, may be a more biologically available source for zinc than those commonly used in swine feeds, and, therefore, provides zinc more readily during the stress of infection.

The disease model chosen to evaluate the stress of disease was swine dysentery. Swine dysentery is an economically important disease of swine. The causative agent has been identified as the bacterium *Serpulina hyodysenteriae*. Because an effective vaccine is not available, management is an important factor in the control of this disease. The cost of this disease to swine producers because of the high mortality rates often associated with this disease, and in the lost weight gain and poor feed efficiency of the survivors. The known virulence factors of *Serpulina hyodysenteriae* are a hemolysin that is cytolytic for a variety of cell types, and the lipopolysaccharide which has endotoxin activity. Pretreatment with zinc has

been shown to enhance survival, or the recovery rate, of other species challenged with various bacteria or viruses. Preliminary results suggested that dietary supplementation of zinc to the diets of young pigs as zinc methionine may reduce the mortality of pigs challenged with *Serpulina hyodysenteriae.*

The main objectives to evaluate the hypotheses of this research were: 1) to determine whether the type of dietary zinc supplement has an effect on the ability of young pigs to resist challenge with *Serpulina hyodysenteriae*, 2) to examine the effects of zinc deficiency on selected immune functions of young swine, 3) to determine whether the type of dietary zinc supplemented has an effect on selected immune responses in young swine, and 4) to examine the effect of zinc on *Serpulina hyodysenteriae* infections using a mouse model.

Explanation Of Dissertation Format

The format for this dissertation includes five manuscripts written in the style of the American Society of Animal Science (Journal of Animal Science). A literature review precedes the first manuscript, and a general summary follows the last manuscript. References for the literature review and the general summary are listed in a literature cited section which follows the general summary. Literature cited in the manuscripts are at the end of each manuscript, and is followed by any tables or figures cited in the text. The appendix contains the analysis of variance tables for data presented in this dissertation.

LITERATURE REVIEW

Zinc is the second most abundant trace element in the body of animals (Widdowson and Dickerson, 1964; Miller et al., 1978). Zinc is distributed in every tissue throughout the body. The prostate and eye have the highest concentrations of zinc, and fat has the lowest concentration (Miller, 1969; Miller et al., 1968; Underwood, 1977). The fungus *Aspergillus niger* was the first species for which a nutritional requirement for zinc was documented (Raulin, 1869). In 1934, zinc was established as an essential nutrient for rats and mice (Bertrand and Bhattacherjee, 1934; Todd et al., 1934).

Zinc affects a diverse number of processes, and a deficiency results in reduced growth rate, dermatitis, deformed bones, slowed development of secondary sex characteristics, slowed wound healing, and impaired immune function in species studied. Keilin and Mann (1940) reported that carbonic anhydrase of erythrocytes contained zinc at 1 g-atom of Zn/mol, and established an essential role for zinc in metalloenzymes as a primary biological function of this element. Zinc metalloenzymes are found among all classes of enzymes (Vallee and Galdes, 1984). Zinc may function as a catalytic component as it does in RNA polymerase I from yeast (Auld and Atsuya, 1976) or, as a structural component, as it does in superoxide dismutase (Galdes and Vallee, 1983), with copper playing the catalytic role in this enzyme. Zinc may also act as a regulator, activating or inhibiting enzyme function without actually being essential for activity as it does in certain hydrolases (Galdes and Vallee, 1983). Cellular concentration of zinc can, therefore, influence many biological functions through the effects on various enzymes, including carbohydrate, fat, and protein metabolism, and nucleic acid synthesis and degradation. A decrease in enzyme activity in response to deficiency depends on how thermodynamically stable the protein-zinc association is and the rate of turnover of the protein.

Zinc serves a structural role in metalloproteins other than enzymes. There are three

distinct motifs of DNA-binding zinc metalloproteins characterized as "zinc finger," "zinc twist," and "zinc cluster" (Vallee et al., 1991). *Xenopus* transcription factor IIIA, a "zinc finger" protein was the first gene regulator shown to be a metalloprotein (Miller et al., 1985). A fungal transcription factor, GAL4, is an example of the "zinc twist" motif (Pan and Coleman, 1990). An example of a "zinc cluster" is the DNA binding domain of the mammalian glucocorticoid hormone receptor (Pan et al., 1990).

Zinc binds to keratin, by partly cross-linking sulphydryl groups and protecting it from oxidative damage (Williams, 1984). This function is important in the tails of sperm, and in hair and nails. Zinc interacts with insulin through stabilizing and polymerization of the prohormone (Kirchgessner and Roth, 1980). The binding of human growth hormone to the prolactin receptor is increased by zinc, with one zinc ion per hormone receptor complex (Cunningham et al., 1990). Besides receptor binding, another possible role for zinc in endocrine function may be an effect on signal transduction, by activation of protein kinase C, and the binding of protein kinase C to membranes (Csermely and Somogyi, 1989).

The fragility of erythrocytes increases in zinc deficiency and is corrected by zinc supplementation, even when there is little change in the zinc concentration of other soft tissues (Bettger and Taylor, 1986; O'Dell et al., 1987; Johanning and O'Dell, 1989). Zinc has been reported to prevent the hemolysis induced *in vitro* by a variety of cytolytic agents (Avigan and Berniheimer, 1976) including the hemolysin of *S. hyodysenteriae* (Hyatt et al., 1992), and complement (Götze et al., 1968; Yamamoto and Takahashi, 1975; Boyle et al., 1979). The ability of extracellular zinc to protect a cell from lysis is about 10 times greater than the effect of calcium (Pasternak et al., 1985; Bashford et al., 1986). The ratio of cholesterol to phospholipid in plasma and microsomal membranes increases with zinc deficiency resulting in decrease in membrane fluidity (Clejan et al., 1981; Dib and Carreau, 1985).

Zinc Requirement of the Pig

The classic sign of zinc deficiency in growing swine is called parakeratosis. The condition is characterized by a hyperkeratinization of the skin, retarded growth, listlessness, vomiting, and diarrhea (Kernkamp and Ferrin, 1953; Tucker and Salmon, 1955). Other effects of zinc deficiency in pigs include reduced rate of gain and feed efficiency, lowered levels of serum alkaline phosphatase, zinc, and albumin (Hoekstra et al., 1956, 1967; Luecke et al., 1957; Theuer and Hoekstra, 1966; Miller et al., 1968, 1970; Prasad et al., 1969, 1971; Ku et al., 1970). Boars fed zinc deficient diets have reduced testicular development (Miller et al., 1968), and have a greater zinc requirement than gilts that, in turn, have a higher requirement than barrows (Liptrap et al., 1970; Miller et al., 1970). A dietary excess or deficiency of zinc results in fewer pigs per litter and smaller pigs (Pond and Jones, 1964; Hoekstra et al., 1967; Hill et al., 1983; Hill and Miller, 1983). Low levels of dietary zinc during the last month of pregnancy prolonged the duration of farrowing in sows (Kalinowski and Chavez, 1984).

A number of dietary factors influence the requirement of the pig for zinc (Miller et al., 1979). A young pig on a casein-glucose diet requires 15 ppm zinc (Shanklin et al., 1968) but, because plant phytates and other dietary factors reduce the bioavailability of zinc, the NRC (1988) recommends 100 ppm of zinc in diets for young pigs. Chelates of zinc, which will release it in ionic form at the intestinal wall, or will be readily absorbed as an intact chelate, can increase the bioavailability of zinc by preventing its conversion to insoluble compounds in the lumen of the intestine. Owen et al. (1973) demonstrated an increase in the bioavailability of zinc when the chelating agent ethylenediamine tetraacetic acid (EDTA) was fed. Histidine has also been shown to enhance absorption of zinc in pigs (Dahmer et al., 1972). Hill et al. (1986) found no difference, however, in the biological value of zinc as zinc sulfate, or chelated with the amino acid methionine with or without picolinic acid.

Zinc Metabolism

The mechanism of zinc absorption is not precisely known. Because zinc complexes so readily with a variety of feed components, the chemical form of zinc that interacts with the intestinal epithelium may influence the pathway(s) by which zinc absorption occurs. Zinc movement across the mucosa of the small intestine is thought to be at least partially achieved by a carrier mediated diffusion mechanism (Pearson et al., 1966; Davies, 1980; Menard and Cousins, 1983; Steel and Cousins, 1985; Song and Adham, 1986; Hoadly et al., 1987) and may involve an absorbable or intracellular ligand (Evans et al., 1975; Cousins, 1985; Hempe and Cousins, 1989). Three suggested ligands for zinc absorption include: prostaglandin E_2 (Song and Adham, 1977), intestinal metallothionein (Cousins, 1978), and cysteine-rich intestinal protein (CRIP, O'Dell, 1992).

Prostaglandin E_2 facilitates zinc absorption *in vivo* and enhances zinc uptake *in vitro* (Song and Adham, 1977). Injections of aspirin depressed zinc absorption and ^{14}C-arachadonic acid administered orally appeared with zinc binding ligand from the rat intestine (Evans and Johnson, 1978). Mooradian and Song (1987) suggest prostaglandins may affect net zinc absorption by inhibiting transport from the serosa to the mucosa. Thus, prostaglandin E_2 may not be a ligand to which zinc is bound and transported across the mucosa, but prostaglandins may be important regulators of net zinc absorption.

The metal binding protein, metallothionein, has a central role in zinc homeostasis (Cousins, 1978). Dietary and plasma zinc concentrations regulate synthesis of the protein (Richards and Cousins, 1976). Hoadley et al. (1988) demonstrated an inverse relationship between the rate of zinc absorption and the intestinal metallothionein concentration, and a direct relationship between the portion of mucosal zinc available for absorption and intestinal metallothionein concentration. Metallothionein may, therefore, act as a mucosal buffer for zinc.

A new zinc carrier protein has recently been isolated and characterized (Hempe and Cousins, 1991, 1992). Hempe and Cousins (1992) have proposed a model for zinc absorption in which CRIP serves as a shuttle for zinc from the apical to basolateral membrane, with metallothionein and nonspecific binding proteins competing with CRIP for zinc in the enterocyte. The model is based on observations of ^{65}Zinc absorption from intestinal loops of rats fed either 1 or 180 mg Zn/kg. As the concentration of zinc increased, the concentration of ^{65}Zinc bound to metallothionein also increased. There was no change in the total zinc bound to CRIP, but there was a change in the proportion of ^{65}Zinc bound to CRIP. This suggests that CRIP exhibits saturation kinetics. Further work is needed on models of zinc absorption.

Once zinc reaches the basolateral membrane it is transferred to proteins in the blood for transport to other tissues. In most species, albumin is quantitatively the most important plasma zinc binding protein, but in the pig alpha$_2$-macroglobulin and another component, thought to be unique to porcine plasma, are of equal importance (Chesters and Will, 1981a). The level of zinc found in tissues is influenced by dietary intake and other factors such as infection or inflammation.

Some characteristic changes occur as a part of the acute phase response when an animal is exposed to infectious or inflammatory agents. Zinc concentration decreases in serum, bone, skin, and intestine (Cousins and Leinart, 1988) and, the zinc content of the liver increases dramatically. The zinc content of thymus, and bone marrow also increases. This change in tissue distribution of zinc results from increases in the tissue levels of metallothionein. Injecting pigs with endotoxin induces a decrease in the plasma zinc concentration (Chesters and Will, 1981b). Injecting rats with interleukin-1 (IL-1) mimics the effects of injecting endotoxin (DiSilvestro and Cousins, 1984). The effect of IL-1 on the liver is to stimulate the synthesis of IL-6 (Dinarello, 1988). Studies using recombinant interleukins and rat hepatocytes support IL-6 as the major mediator of metallothionein induction, at the level of the liver cell. When rhIL-6

was added to cultures of hepatocytes the levels of metallothionein mRNA, and the metallothionein protein, increased within 3 hours. The addition of rhIL-1 did not increase metallothionein (Dinarello, 1988).

It is not known whether the reduction in plasma zinc and loading of the tissues results in an increase in the dietary requirement for zinc during these periods of stress. Zinc deficient pigs are more susceptible to enteric infections than control pigs; and enteric infection enhanced the production of zinc deficiency (Whitenack et al., 1978). A recent study in cattle infected with infectious bovine rhinotracheitis virus indicates that dietary zinc enhances the rate of recovery from that viral infection (Chirase et al., 1991).

Immune System Overview

The role of the immune system is to defend the integrity of the host by distinguishing self from nonself, then appropriately eliminating foreign materials. To complete this task, a variety of defense mechanisms have evolved. These mechanisms are divided into native or nonspecific immunity, which act as a first line of defense against infectious agents, requiring no previous exposure to antigen, and acquired, or specific immunity, which is antigen driven and requires two or more weeks to detect a response to an initial exposure. Acquired immunity has memory and is able to respond more rapidly upon subsequent exposures to the same antigen. Although the functions of the native and acquired immune systems are separated for the purpose of studying immune responses, they interact to provide an integrated defense.

The components of the native immune system include physical barriers such as the skin, and the gastrointestinal wall and motility. Chemical and antimicrobial barriers to infection include, but are not limited to, the mucous layer, normal microflora, bactericidal fatty acids, pH, and enzymes. Other components of this system include complement, phagocytic cells, natural killer cells, and cytokines.

The skin and the mucosal membranes form a mechanical barrier to the environment and are the first place of contact for most antigens. The outer layer of the skin, the stratum corneum, is made up primarily of a structural protein, keratin (Shonle, 1988). The cells of the stratum corneum have lost their nuclei, become flattened and are continually shed. Most microorganisms cannot use keratin as a nutrient source, so the life span of these organisms on the skin is limited (Shonle, 1988). The continual shedding of the stratum corneum assists in the removal of organisms that cannot invade beyond the skin surface. In addition to the physical barrier, the sebaceous glands secrete sebum that contains fungistatic lipids, saturated fatty acids with odd numbered carbon chains from C7 to C11 (Rothman et al., 1947; Kligman and Ginsberg, 1950; Abraham et al., 1975).

The mucosal surface also provides many barriers to organisms in the environment. Besides the barrier that the epithelium provides, there are physical barriers, such as the mucociliary system in the respiratory tract, to remove particulate antigen (Kuper et al., 1992), and the peristaltic action of the gastrointestinal tract. Chemical barriers include the acidic conditions of the stomach, and the enzymes of the intestinal tract. The mucus layer is also an important barrier that can mechanically trap organisms. The trapped organisms may be physically removed, or may interact with antibodies, or enzymes, present in the mucus layer.

The complement system is an important factor in controlling bacterial infection and mediating inflammation. Some of the biological activities of the complement system includes: vasodilation, increased vascular permeability, phagocytic chemotaxis, opsinization, and destruction of cell membranes (Osler, 1976; Kinoshita, 1991). The complement system is composed of serum proteins that are activated in an enzyme cascade system. This system provides for an antibody specific (classical pathway) and a non-specific (alternative pathway) mechanism for recognizing foreign agents. The classical pathway uses nine major proteins, and is triggered when C1q binds to the Fc portion of an antibody-antigen complex. The

alternative pathway uses six major proteins, and is triggered by bacterial products and proteases released by damaged tissue. Components of the two pathways are listed in Table 1. All of the major proteins of the complement system have been identified in swine sera (Barta and Hubbert, 1978). Besides the major proteins, there are many regulatory proteins associated with the complement system that keep it from damaging host tissues (Osler, 1976; Kinoshita, 1991).

Table 1: Complement proteins of the Classical and Alternative Pathways

Classical Pathway	Alternative Pathway
C1q, C1r, C1s	C3
C2	C5
C3	C6
C4	C7
C5	C8
C6	C9
C7	
C8	
C9	
Regulatory proteins	
Properdin	Factor B
C1 inhibitor	Factor D
Factor I	Properdin
C4-binding protein	C3b inactivator
(C4bp)/Factor H	ß1H
S protein	
Sp40/40	
Decay-accelerating factor (DAF)	
Membrane cofactor protein (MCP)	
Membrane attack complex inhibition factor (MACIF)	

Phagocytic cells engulf and destroy microorganisms. There are two major types of phagocytic cells: polymorphonuclear phagocytes, and mononuclear phagocytes. The polymorphonuclear phagocytes include the neutrophils, eosinophils and basophils. Mononuclear phagocytes include the macrophages and monocytes. Phagocytes are produced in the bone marrow. The blood stream carries phagocytes to the tissues where they function.

Neutrophils are attracted to the site of infection by chemotactic substances produced by

the microorganisms, generated by cleavage of complement components, or released by lymphocytes. Once at the site, neutrophils ingest particles into a membrane bound vesicle called a phagosome. The granules or lysosomes, in the cytoplasm of the neutrophil, fuse with the phagosome. Hydrolytic enzymes and other bactericidal substances are released into the phagosome, resulting in the destruction of the microorganism. Neutrophils also perform antibody-dependent, cell-mediated cytotoxicity functions. This is a mechanism that allows destruction of a virus infected cell. Antibody binds to the virus infected cell and the neutrophil. The neutrophil then destroys the infected cell.

Macrophages may be specialized cells fixed in tissues (i.e., Langerhans' cell of skin and Kupffer cell of the liver), or they may wander like neutrophils, migrating to infected tissues (Hume and Doe, 1988). Fixed macrophages are important for clearing immune complexes and removing antigens from the blood and lymph. Wandering macrophages perform functions similar to neutrophils. In addition to phagocytic functions, macrophages present antigens to T lymphocytes and are thus an important bridge between the non-specific and specific immune system (LaFuse and David, 1984).

Lymphocytes and their products are the major components of the acquired immune response. There are two major classes of lymphocytes: T cell, thymus-processed lymphocytes, and B-cells, bursa-derived for avian lymphocytes, or bone marrow-derived for mammals. The two main functions of T cells are the destruction of host cells that express foreign antigens and the regulation of the immune response through the secretion of lymphokines.

Lymphocytes in pigs vary significantly from other mammals. The peripheral blood lymphocyte count is about 10^7/mL, which is twice the concentration of lymphocytes in cattle or sheep (Coles, 1974). Nearly half of the circulating lymphocytes are null cells that do not express markers for T or B cells (Duncan et al., 1989). The functional role of these null cells

is unknown (Duncan et al., 1989).

T cells that directly attack host cells expressing foreign antigens are referred to as cytotoxic T cells (T_c cells). T_c cells only recognize antigen associated with class I major histocompatability complex (MHC) molecules. Most T_c cells express the Cluster of Differentiation (CD) 8 marker on their surface.

Enhancement of the immune response is performed by helper T cells (T_h cells), which recognize antigen in association with the class II MHC molecules. Antigen presenting cells present foreign antigens in conjunction with class II MHC to the T_h cells. If interleukin-1 (IL-1) is also present, T cells become activated and secrete IL-2 and other lymphokines, important in enhancing B cell and other T cell responses. T_h cells usually express the CD4 marker on the cell surface. In human peripheral blood the ratio of CD4 to CD8 cells is normally 1.5 to 2.0, but in the pig the CD4 to CD8 ratio is 0.6 (Pescovitz et al., 1984, 1985). About 25% of the swine peripheral T cells express both CD4 and CD8 (Pescovitz et al., 1985); this occurs only in pathological conditions in man. The population of T cells expressing both CD4 and CD8 may be memory T cells in the pig (Zuckerman and Husman, 1992). A third unusual characteristic of swine is that CD8 cells constitutively express class II MHC antigen (Lunney and Pescovitz, 1987). It is not known why the pig has a higher percentage of CD8 positive cells or why these cells express class II MHC antigens, but because the pig is capable of mounting normal immune responses these differences do not appear to be detrimental.

The main characteristic of the B lymphocyte is the ability to synthesize immunoglobulin. Synthesized immunoglobulin may remain in the cytoplasm, be incorporated into the cell membrane, or secreted into body fluids as antibodies. Five classes of immunoglobulin (Ig) have been identified in humans and mice, and are referred to as IgM, IgG, IgA, IgE, and IgD. Only three classes of immunoglobulin, IgG, IgM, and IgA, have been demonstrated in the pig (Porter, 1969). Eighty percent of the immunoglobulin found in

porcine serum and colostrum is IgG (Porter, 1969; Curtis and Bourne, 1971). There are two main subclasses of porcine IgG identified as IgG_1 and IgG_2 (Metzger and Fougereau, 1968). Two additional classes of IgG, IgG_3 and IgG_4, are also present in serum but in much lower quantities (Metzger and Fougereau, 1968). An immunoglobulin antigenically similar to IgG with a sedimentation constant closer to IgM has been described, and suggested to be the true primary response immunoglobulin in the pig (Kim et al., 1966; Porter, 1969). There is also a low molecular weight IgG found in serum of newborn and germ free pigs that may be missing the light chain and would, therefore, not be functional (Stertzl et al., 1960; Franek et al., 1961; Franek and Riha, 1964). IgM is the least abundant of the three classes of immunoglobulins and accounts for about 5% of the total immunoglobulin in serum and colostrum (Porter, 1969; Curtis and Bourne, 1971). It is important in the serologic response to gram negative bacteria (Franek et al., 1962; Rowley and Turner, 1964) and plays a complementary role to IgA in the local immune response in the gut. Only low levels of IgA are detected in the serum or colostrum but, dimeric IgA, with a J chain and associated secretory component, are the predominate class of immunoglobulin in gut secretions (Porter and Allen, 1972).

Other features of the pigs immune system that varies from that of humans is in the structure of the lymph nodes and the circulation of the lymphocytes. The lymph nodes of pigs are structurally inverted compared to other domestic animals (Anderson, 1972). The lymph from these nodes is nearly acellular; the cells leave the node and enter directly into the blood (Binns et al., 1986). Other organs such as the spleen, thymus and Peyers patches are structurally similar to other mammals (Binns et al., 1986; Pabst and Binns, 1986).

Effect of Zinc on Immune Function

Zinc is widely accepted as an important nutritional factor for maintaining the immune functions of man and animals. Miller et al. (1968) observed that zinc deficient pigs have

significantly reduced thymus weights and elevated leukocyte counts. The percentage of lymphocytes in these pigs decreased and the immature neutrophil population increased (Miller et al., 1968). A decrease in the percentage of lymphocytes was also found in zinc deficient rats (Dreosti et al., 1968; Macapinlac et al., 1966). Experimental investigations of the interrelations between zinc and immune function, using a murine model, have been carried out in a number of laboratories. These investigations demonstrate an influence of zinc on many factors important to host defense mechanisms. Zinc deficiency results in rapid involution of the thymus (Haas et al., 1976; Frost et al., 1977; Miller, 1968; Quarterman, 1974), loss of T cell functions (Fraker et al., 1977), reduction in antibody production (Luecke et al., 1978), a drop in thymic hormone levels (Bach et al., 1975), loss of Thy-1 positive lymphocytes (Bach et al., 1975), and reduced IL-1 and IL-4 activity (Winchurch et al., 1987). Besides a general suppression in antibody production, zinc seems to be important in class switching from IgM to IgG or IgA immunoglobulin production (Nash et al., 1979). The depressed thymic hormone levels, thymic atrophy and T cell dysfunction precede the weight loss and acrodermatitis associated with zinc deficiency (Nash et al., 1979).

Zinc supplementation increased IL-1 approximately 300% over unsupplemented controls, and enhanced the ability of concanavalin A activated T cells, from aged mice, to produce B cell stimulatory factor 1 (IL-4) (Winchurch et al., 1987). Lead, nickel, and zinc induced the proliferative response of murine splenocytes (Warner and Lawrence, 1986). This increase in proliferative capacity required the presence of T cells and Ia positive cells (Warner and Lawrence, 1986). Zinc supplementation of leukocyte cultures, *in vitro*, increased the production of tumor necrosis factor (TNF) and IL-1ß but, had no effect on the production of IL-6 (Scuderi, 1990). Murine thymocyte cultures supplemented *in vitro* with zinc had an enhanced proliferative response compared to unsupplemented cultures when stimulated with IL-1 (Winchurch, 1988). Zinc affected the early stages of the proliferative response. This

suggests that zinc may enhance the cellular uptake of IL-1 or facilitate the steps subsequent to IL-1 binding (Winchurch, 1988). James et al. (1987) demonstrated that the impaired mitogenesis observed in zinc deficient mice, after six weeks of zinc depletion, is due to a primary defect in the macrophage population in culture; however, after twelve weeks of zinc depletion, the proliferative capacity of T cells was depressed, suggesting a direct effect on the T cell subpopulations. T cells, from zinc deficient and normal rats, behave normally in presence of normal rat serum, but when cultured with zinc deficient rat serum, there is an impaired proliferative response (Dowd et al., 1986).

The stage of the animal's development is an important determinant associated with the effect of zinc deprivation on the immune response (Beach et al., 1980a). Even marginal zinc deficiency resulted in depression of T cell responses, when instituted in fetal or early postnatal life (Beach et al., 1980b). Maternal deprivation during gestation resulted in an immunodeficiency in the newborns that persisted for three generations in mice (Beach et al., 1982).

Moderate zinc deficiency, in rhesus monkeys, resulted in a decreased chemotactic response to formyl-methionyl-leucyl-phenylalanine. Moderate zinc deficiency did not inhibit the phagocytic capability of the neutrophils (Vruwink et al., 1991). The chemotactic deficiency was not corrected by *in vitro* supplementation of zinc, but was corrected by dietary repletion of zinc for two weeks (Vruwink et al., 1991). Neutrophil function, in humans, may also be depressed by excess zinc (Chandra, 1984).

The classical pathway of complement activity is enhanced by zinc in the presence of optimal calcium and magnesium (Amiraian et al., 1974). The enhancement of the classical pathway by zinc is due to an increase in C5 activity (Montgomery et al., 1979; Evans and Amiraian, 1984). This increase results from the activation of C5 by C5 convertase, when zinc is at an optimal concentration of 0.025 mM (Evans and Amiraian, 1984). At a concentration of

.1 mM, zinc blocks the activity of C3b inhibitor (Crossley and Porter, 1980). Zinc and magnesium together enhance the lysis of sheep erythrocytes by cobra venom factor treated serum and guinea pig serum deficient in the fourth component of complement. The enhanced lysis suggests that zinc and magnesium together activate the alternative pathway of complement on a nonactivator surface (e.g., the sheep red blood cell, Evans and Amiraian, 1987).

Swine Dysentery: An Overview

Swine dysentery is a mucohemorrhagic diarrheal disease, most commonly observed in pigs at about 8 to 12 weeks of age, but may affect weanling to adult animals. This disease was reported to be present in most swine producing countries of the world (Roncalli and Leaning, 1976), and continues to be a problem for swine producers throughout the world. The cost to swine producers due to death of pigs, decreased rate of growth, loss of feed efficiency, and expenses for chemotherapy is estimated at 100 million dollars annually (Owen, 1986). In outbreaks of swine dysentery in weanling pigs, the morbidity is usually over 90 percent and mortality may reach 30 percent.

Swine dysentery was originally described by Whiting et al. (1921), but the primary etiologic agent was not identified until 50 years later when researchers at Cambridge University isolated and propagated a pathogenic anaerobic spirochete (Taylor and Alexander, 1971). This spirochete was confirmed as the causative agent of swine dysentery (Glock and Harris, 1972; Harris et al. 1972), and the organism was named *Treponema hyodysenteriae*. The organism was recently reclassified as a new genus, *Serpulina*, based on a lack of homology of 16s ribosomal RNA between *T. hyodysenteriae* and *Treponema pallidum*, the type species of the genus (Stanton et al., 1991; Stanton, 1992). Although *Serpulina hyodysenteriae* is the primary agent of swine dysentery, other microorganisms indigenous to the intestinal tract may contribute to the lesions observed during the course of the disease (Meyer et al., 1974ab, 1975;

Harris et al., 1978; Whipp et al., 1979; Joens et al., 1981).

The disease is transmitted primarily by ingestion of fecal material, either from clinically affected pigs or from asymptomatic carrier animals. Introduction of the disease into a herd may also occur on the footwear or clothing of animal caretakers. Outbreaks of the disease can usually be explained by the introduction of carrier animals into a herd; but other vectors such as dogs, mice, birds, and flies have been implicated in the spread of the organism (Songer et al., 1978; Glock et al., 1978; Songer and Harris, 1978; Joens and Kinyon, 1982). Some stressors that may contribute to the induction of symptoms include: changing feed, shipping animals, over crowded conditions, exposure to low environmental temperatures, and castration. Because the organism is able to survive under a wide range of environmental conditions (Glock et al., 1975; Chia and Taylor, 1978; Egan, 1981), may be shed for 70 days from recovering pigs with no clinical signs of disease (Songer and Harris, 1978), and may even be shed in asymptomatic pigs with no serologic response to the organism (Hampson et al., 1992), it is difficult to control the spread of this organism. Because no effective vaccine is available, good management practices are essential in controlling the spread of this disease.

The incubation period of swine dysentery varies from 2 days to 3 months, but usually occurs within 2 weeks in naturally exposed pigs. Diarrhea is the most consistent sign of the disease and may vary from a soft yellow to gray colored feces to watery stools containing blood and mucus. If diarrhea is prolonged, the animals become gaunt, weak, uncoordinated, and emaciated. The pigs may die of dehydration, acidosis, and hyperkalemia that are the result of prolonged diarrhea. Occasionally, peracute deaths occur, the cause of which is unknown.

The pathogenesis of swine dysentery begins with the proliferation of *S. hyodysenteriae* in the large intestine of the pig. The spirochetes have been observed in the cytoplasm of enterocytes; but invasion is not believed to be necessary for lesion production (Taylor and Blakemore, 1971; Teige et al., 1981; Albassam et al., 1985; Kennedy et al., 1988). The

mechanism of tissue destruction is not fully understood, although two toxins that may play a role in lesion development have been described. The first of these toxins is a hemolysin that is cytolytic for a variety of cell types including porcine lymphocytes (Kent and Lemcke, 1984) and porcine epithelial cells (Lysons et al., 1991). The *S. hyodysenteriae* hemolysin has been described as a lipoprotein associated with nucleotides; it is oxygen stable, heat labile, and stable over a pH range of 2 to 10 (Saheb et al., 1980). The hemolysin is carrier-dependent (Lemcke and Burrows, 1982), and is active between the temperatures of 20° and 40° C. Studies on the lytic mechanism of this hemolysin on red blood cells indicate that it does not require divalent cations and is not lipolytic or proteolytic (Saheb et al., 1980). The reaction of the hemolysin with the erythrocyte surface is temperature independent, but hemolysis is temperature dependent (Saheb and Lafleur, 1980). Lysis of the target cell appears to be associated with a swelling of the cell; however, release of some hemolysin has been blocked by sucrose (Saheb and Lafleur, 1980). On the basis of these results, Saheb and Lafleur (1980) postulated that the lysis of target cells is caused by a digestion or disruption of components of the cell membrane, which results in a change in membrane permeability. Recently, Hyatt et al. (1992) demonstrated inhibition of the hemolysin by serum lipids, zinc, and copper at a concentration of 5 mM, and is partially inhibited by calcium. Lytic activity of the hemolysin is partially inhibited by compounds with the diameter of 1.0 to 1.1 nm (PEG 1000 or Dextran 1500) suggesting that the mode of action is porin related (Hyatt et al., 1992).

The lipopolysaccharide (LPS) of *S. hyodysenteriae* was originally described by Baum and Joens in 1979. The LPS has endotoxin activity and may have an effect on the epithelial cells in the large intestine of pigs (Nuessen et al., 1983). Greer and Wannemuehler (1989ab) demonstrated that the LPS would stimulate IL-1 and tumor necrosis factor (TNF), which elicit an inflammatory response.

Serpulina hyodysenteriae does not invade beyond the lamina propria of the large

intestine and significant lesions are not found in other organs (Kinyon et al., 1980). Therefore, the pathogenesis of swine dysentery is attributed directly to the enteric lesions (Kinyon et al., 1980). The systemic effects of the disease are the result of fluid and electrolyte imbalance induced by the enteritis. The fluid loss appears to be the result of malabsorption due to the failure of the epithelium to actively transport sodium and chloride from the lumen of the colon into the blood.

Besides conventional pigs, other animal models have been used to study the pathogenesis of *S. hyodysenteriae* infections. These other models include; gnotobiotic swine (Meyer et al., 1974ab, 1975; Harris et al., 1978; Joens et al., 1981; Whipp et al., 1979, 1982), chicks (Sueyoshi, and Adachi, 1990), guinea pigs (Joens et al. 1978), and various strains of mice (Joens and Glock, 1979; Nibbelink and Wannemuehler, 1991, Nibbelink, 1992; Suenaga and Yamazaki, 1983).

Gnotobiotic pigs have been used to study the interaction between other bacteria and *S. hyodysenteriae* in the production of lesions. The initial indication that other bacteria might be important in the development of clinical signs of swine dysentery was made by Meyer et al. (1974a) when germ free pigs were challenged with *S. hyodysenteriae* B78, *Vibrio* (Campylobacter) *coli*, a combination of the two bacteria, or minced gut scrapings from swine with clinical disease. Only those pigs fed the gut scrapings exhibited clinical signs of swine dysentery. This led Meyer et al. (1974a) to suggest that *S. hyodysenteriae* might require another synergistic organism to bring about the disease state. Not all bacteria can serve as a synergistic organism for *S. hyodysenteriae*. No synergistic effect was found by coinfections with *Vibrio coli* (Meyer et al., 1974a), *Escherichia coli*, *Lactobacillus*, *Campylobacter coli* or *Clostridium* (Meyer et al., 1974b). Coinfection of *S. hyodysenteriae* with four uncharacterized anaerobes (Meyer et al., 1975), *Bacteriodes vulgatus*, or *Fusobacterium necrophorum* (Harris et al., 1978) did lead to clinical disease.

The first use of mice as a model for the study of swine dysentery was by Joens and Glock (1979). Mice challenged in that study developed mucoid feces; when the mice were necropsied, macroscopic and microscopic lesions were found in the cecum and colon (Joens and Glock, 1979). A variety of strains of mice have been studied as possible models for *S. hyodysenteriae* infections including: CF-1 (Joens and Glock, 1979), C3H/HeJ, C3H/HeN, Balb/cByJ (Nibbelink and Wannemuehler, 1991), athymic nude mice (Suenaga and Yamazaki; 1983), and SCID mice (Nibbelink, 1992). Nibbelink and Wannemuehler (1991) demonstrated the importance of the host response to LPS for the development of lesions present in *S. hyodysenteriae* infections. They found that C3H/HeJ mice, that are hyporesponsive to LPS, were less susceptible to lesion development than the C3H/HeN mice, which are responsive to LPS. By using these, and other mouse strains with varying immune response criteria, investigations into virulence factors, and the immune responses to these factors, may lead to a better understanding of the pathogenesis of swine dysentery, and the development of a method to prevent or control this disease.

PAPER 1: EFFECT OF ZINC LEVEL AND SOURCE ON THE PATHOGENESIS OF SWINE DYSENTERY IN WEANLING PIG

ABSTRACT

The purpose of this study was to determine the effects of the level and source of supplemental Zn on the clinical signs of swine dysentery in young pigs. Sixteen weanling crossbred pigs, four pigs per treatment, were fed a corn and soybean meal diet supplemented with trace mineral premixs formulated to provide either 40 or 100 ppm Zn. The zinc was supplied as either ZnO or zinc methionine (ZnMet). Chemical analysis of the dietary concentrations of zinc in the 40 ppm diets were 91 and 86 mg/kg, and in 100 ppm diets were 118 and 111 mg/kg (ZnO and ZnMet, respectively). The experimental period was a total of 5 wk (2 wk prior to and 3 wk following challenge). The pigs were challenged on two consecutive days, and then monitored for 19 d following challenge. During the 2 wk before infection, there was no effect of dietary treatment on feed intake or weight gain. After 2 wk of dietary treatment, pigs fed 100 ppm supplemental Zn had higher levels of zinc in the plasma than did pigs fed the diets supplemented with 40 ppm Zn ($P < .03$). Plasma zinc levels of pigs fed the ZnMet supplement were higher after 2 wk of treatment than those of pigs fed the ZnO supplement ($P < .05$), with pigs fed 40 ppm ZnMet having plasma levels similar to those of pigs fed 100 ppm ZnO (.85 and .84 ppm, respectively). Severe clinical signs of disease were observed in 15 of 16 pigs by 5 d after challenge. The clinical signs of disease observed during the first 5 d following challenge were selected as indicators of susceptibility to disease. Clinical scores tended to be lower in pigs fed ZnMet compared with pigs fed ZnO at 3 d ($P < .06$) and 4 d ($P<.08$) after challenge. There was a significant interaction between the level of Zn and the source of Zn 3 d after challenge for clinical signs of disease ($P < .05$). Pigs fed ZnMet at 40 ppm had the lowest clinical scores with a mean of 2.5 (3 d). Pigs fed ZnMet consumed more feed for the first week following challenge than pigs fed ZnO ($P < .02$). The overall mortality rate for this experiment was 7 of 16 pigs at 19 d with a trend for less mortality in pigs fed ZnMet (2 of 8) compared to pigs fed ZnO (5 of 8, $P <.057$). Pigs fed ZnMet had greater levels of Zn in plasma prior to challenge compared with pigs fed ZnO, and tended to have fewer clinical signs of disease for the first 5 d after challenge. The level of zinc supplemented did not have an effect on clinical

signs of disease. There may be some advantage to ZnMet as a supplement during the stress of disease.

Key words: Zinc, Swine dysentery, Zinc methionine

INTRODUCTION

Administration of zinc has been shown to enhance the survival of rats infected with *Salmonella typhimurium* (Tocco-Bradley and Kluger, 1984), *Francisella tularensis* or *Streptococcus pneumoniae* (Sobocinski et al., 1977). All zinc deficient pigs died following an intraperitoneal injection with *Salmonella pullorum*, but there was no mortality among pigs receiving a zinc adequate diet (Miller et al., 1968). Recent studies, investigating the effect of zinc methionine and zinc oxide on cattle challenged with infectious bovine rhinotracheitis virus indicated that dietary zinc supplied as zinc methionine was more effective in enhancing recovery from infection (Chirase et al., 1991). This indicated that the source of zinc may vary in the ability to provide adequate zinc during stress situations. Preliminary studies, conducted at the Veterinary Medicine Research Institute, Iowa State University, suggested that supplementing the diet of young swine with zinc methionine improved the survival rate and feed efficiency of pigs infected with *Serpulina hyodysenteriae*. The improvement in performance was not observed when the rations were supplemented with zinc oxide (Wannemuehler and Crump, personal observation). There was no difference in the occurrence of swine dysentery between pigs fed rations supplemented with zinc oxide or zinc methionine.

The objective of this study was to determine whether the level or source of zinc supplemented in corn and soybean meal rations have an effect on the severity of clinical signs of swine dysentery in young pigs.

MATERIALS AND METHODS

Sixteen, Landrace x Large White x Duroc, crossbred pigs were weaned at 3 to 4 wk of age (average initial weight, 9.7 kg). The pigs were housed individually in modified 1 m x 1 m cages in two rooms at the Veterinary Medical Research Institute, Iowa State University. The pigs were grouped by initial weight in a randomized block design, and treatments were assigned randomly to a pig in each block. Each pen was equiped with either a stainless steel or plastic self-feeder and a nipple waterer. Pigs were allowed ad libitum access to feed and water for the 2 wk before challenge and for the 3 wk following challenge. Feed was withheld during the 2 day challenge. The dietary treatments consisted of a corn and soybean meal basal diet supplemented with trace mineral premixes formulated to supply either 40 or 100 ppm zinc. Zinc was supplied as either zinc oxide (ZnO) or zinc methionine (ZnMet, Table 1.1). Although the diets were formulated to have two levels of zinc differing by 60 ppm the analyzed content of zinc varied by only 25 to 27 ppm. The reason for this discrepency is unclear. Feed intake and body weight of each pig were monitored weekly.

Challenge organism. The organism used in this trial, *Serpulina hyodysenteriae* strain B204, was grown as previously described (Nibbelink and Wannemuehler, 1991). Briefly, *S. hyodysenteriae* were grown anaerobically at 37°C in Trypticase Soy Broth (BBL Microbiological Systems, Cockeysville, MD) supplemented with 5% horse serum (Hyclone Laboratories, Logan UT), .5% yeast extract (BBL Microbiological Systems), and 1% VPI salt solutions (Solution A: $CaCl_2$, .4 g/L, and $MgSO_4$, .4 g/L; Solution B: $KHPO_4$, 2 g/L; KH_2PO_4, 2 g/L; $NaHCO_3$, 20 g/L; NaCl, 2 g/L). Log phase cultures were obtained. Prior to inoculation of the pigs, the numbers of bacteria were determined using a Petroff-Hauser counting chamber, and the bacteria were diluted to 10^7 organisms per mL with warm complete media.

Challenge procedure. Pigs were challenged twice, 24 hours apart, by gastric intubation of a volume of 100 mL culture broth (10^9 total organisms). Feed was removed the night before administration of the first dose of challenge innoculum and, returned 4 h after the second

administration of challenge innoculum. No feed was available for approximately 44 h. Pigs were monitored daily after challenge for clinical signs of disease. A clinical score of 0 to 6 was assigned to each pig daily. Scores were assigned for consistency of feces 0 = normal to 3 = watery diarrhea. Additional points were added to the score for fecal consistency when blood or mucus were present in feces (1 point each). Thus, an animal with a consistency score of 2 with blood and mucus in the feces would have a total clinical score of 4. Animals found moribund or dead were assigned a score of six.

Plasma zinc determination. Heparinized whole blood was collected weekly, via the orbital sinus. The blood was centrifuged at 500 x g. The plasma was removed and recentrifuged to remove any remaining cells. The plasma was stored at -20°C until plasma zinc determinations were made. The plasma was diluted 1 part plasma to 3 parts distilled water. The diluted samples were then analyzed by flame atomic absorption spectrophotometery (Perkin Elmer, Model 3100, Norwalk CT).

Serum glutathione peroxidase. Blood was collected weekly, via the orbital sinus. The blood was allowed to clot and serum was removed. Serum glutathione peroxidase (GSH Px) was determined by the method of Paglia and Valentine (1967).

Data analysis. Data were analyzed by analysis of variance. The model included dietary treatment and block as the main effects. Single df contrasts were made for type of supplement (ZnO vs ZnMet), level of supplementation (40 ppm vs 100 ppm) and type of supplement X level of supplement interaction. Data are reported as least squares means. Statistical analyses were performed using the General Linear Model of SAS (1989). Because of the mortality rate, statisitical comparisons of pig performance, plasma zinc, and clinical signs of disease for the 4th and 5th wk could not be made.

RESULTS

During the 2 wk prior to challenge with *S. hyodysenteriae*, there were no significant differences in feed consumption, weight gain, or activity of GSH Px (Table 1.2). The pigs fed diets supplemented with 40 ppm zinc had lower plasma zinc during the second week of treatment ($P < .03$, Table 1.2) than pigs fed diets supplemented with 100 ppm zinc. Pigs fed ZnO had lower plasma zinc values than pigs fed ZnMet ($P < .05$) during the second week of the experiment.

During the third week of the experiment (first week following challenge), the feed intake of pigs fed ZnMet diets was greater than that of pigs fed ZnO ($P < .02$, Table 1.3). Pigs fed diets supplemented with 40 ppm ZnMet had the highest concentration of plasma zinc (Table 1.3). Plasma zinc levels tended to be less for the ZnO group compared with the ZnMet group during the third week of treatment (first week after challenge, $P < .1$, Table 1.3). Both the level and source of zinc were important effects on plasma zinc levels, as indicated by the source by level interaction ($P < .055$). Pigs fed ZnMet at 40 ppm had the highest concentraton of plasma Zn 1 wk following challenge (Table 1.3).

Because 15 of 16 pigs had clinical signs of disease by 5 d after challenge (Table 1.4), the period from 1 to 5 d was chosen as the indicator for susceptibility to disease. Pigs fed the ZnMet supplemented diets tended to have fewer symptoms on 3 d ($P< .06$) and 4 d ($P < .08$) following challenge than pigs fed ZnO supplemented diets (Table 1.5). Pigs fed ZnMet at 40 ppm had the lowest clinical scores 3 d and 4 d following challenge (Table 1.5). By 5 d after challenge, 4 of the 16 pigs had died (Table 1.4). The mortality rate for the entire observation period was 7 of 16 pigs at 19 d with a trend for less mortality in pigs fed ZnMet (2 of 8) compared to pigs fed ZnO (5 of 8, $P <.057$). The difference in mortality rate between the two sources of zinc was not statistically significant. Because of the mortality rate, data for pig performance and plasma zinc are not presented for 4 and 5 wk.

DISCUSSION

The trend for pigs fed 100 ppm supplemental zinc to have higher plasma zinc levels may indicate a marginal zinc deficiency in the pigs receiving 40 ppm supplemental zinc. During the 2 wk prior to challenge, no differences were detected due to dietary source or level of zinc on feed consumption, weight gain, or GSH Px activity (Table 1.2). If a moderate zinc deficiency was present it was not severe enough to effect pig performance.

Mansson (1964) observed that pigs with *Clostridium perfringens* infection developed more severe lesions of parakeratosis than did uninfected pigs. Pigs infected with transmissible gastroenteritis virus (TGEV) fed zinc adequate diets had reduced plasma zinc levels in comparison with uninfected controls. The enteric infection produced by TGEV exacerbated the onset of zinc deficiency (Whitenack et al., 1978). No signs of dermatitis were observed at any time during this experiment. The levels of zinc fed were apparently high enough to prevent the skin lesions associated with zinc deficiency, even with the added stress of challenge.

Pigs fed ZnMet tended to have lower clinical disease scores for the first 5 d after challenge. The ability of pigs fed ZnMet to maintain their weight, or continue to have slight weight gains, also indicates that these pigs had a less severe disease during the first week after challenge. Plasma zinc concentrations were higher one week following challenge for pigs fed ZnMet than pigs fed ZnO. One effect of endotoxin during a bacterial infection is a reduction in the level of zinc in serum (Cousins and Leinhart, 1988). It cannot be determined from these data whether the plasma zinc levels are beneficial in reducing the severity of disease or are the result of less severe disease.

Under the conditions of this experiment, ZnMet seemed to have some beneficial effects compared with ZnO during the first 5 d after challenge with *S. hyodysenteriae*. The benefits of ZnMet resulted in reduced clinical signs of disease, maintanence of body weight, improved feed intake and higher levels of Zn in plasma. This did not result in protection from disease because all pigs developed clinical signs of disease during the 19 d following challenge.

IMPLICATIONS

The cost of swine dysentery to producers is in mortality, reduced gain and feed efficiency. ZnMet did not protect pigs from clinical disease in this study but pigs fed this supplement did seem to have less severe disease, improved feed intake, and less weight loss for the first few days after infection. Zinc methionine supplement may be a tool for reducing some of the costs to producers associated with lost gain and feed efficiency during outbreaks of enteric disease such as swine dysentery.

LITERATURE CITED

Chirase, N. K., D. P. Hutcheson, G. B. Thompson. 1991. Feed intake, rectal temperature, and serum mineral concentrations of feedlot cattle fed zinc oxide or zinc methionine and challenged with infectious bovine rhinotracheitis virus. J. Anim. Sci. 69: 4137.

Cousins, R. J., and A. S. Leinhart. 1988. Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin-1. FASEB J. 2: 2884.

Mansson I. 1964. The intestinal flora in pigs with parakeratosis in swine. I. The intestinal flora with special reference to an atypical *Clostridium perfringens* and clinical observations. Acta Vet. Scand. 5: 279.

Miller, E. R., R. W. Luecke, D. E. Ullrey, B. V. Battzer, B. L. Bradley, and J. A. Hoeffer. 1968. Biochemical, skeletal, and allometric changes due to zinc deficiency in the baby pig. J. Nutr. 95: 278.

Nibbelink , S. K., and M. J. Wannemuehler. 1991. Susceptibility of inbred mouse strains to infection with *Serpula (Teponenema) hyodysenteriae*. Infect. Immun. 59: 3111.

Pagalia, D. E., and W. N. Valentine. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70: 158.

SAS. 1989. SAS/STAT® Users Guide: Statistics (Release 6.06) SAS Institute. Inc., Cary NC.

Sobocinski, P. Z., W. J. Canterbury, and M. C. Powanda. 1977. Differential effect of parenteral zinc on the course of various bacterial infections. Proc. Soc. Exp. Biol. Med. 156: 334.

Tocco-Bradley, R., and M. J. Kluger. 1984. Zinc concentration and survival in rats infected with *Salmonella typhimurium*. Infect. Immun. 45: 332.

Whitenack, D. C., C. K. Whitehair, and E. R. Miller. 1978. Influence of enteric infection on zinc utilization and clinical signs and lesions of zinc deficiency in young swine. Amer. J. Vet. Res. 39: 1447.

Table 1.1: Composition of Experimental Diets as a Percentage

Ingredient	Zn Oxide 40	Zn Oxide 100	Zn Met 40	Zn Met 100
Ground Corn	71.52	71.52	71.42	71.27
Soybean meal	25.00	25.00	25.00	25.00
Dicalcium phosphate	1.28	1.28	1.28	1.28
Calcium carbonate	.90	.90	.90	.90
Vitamin-corn premix[a]	1.00	1.00	1.00	1.00
Mineral premix[b]	.30[c]	.30[d]	.30	.30
ZINPRO 40[e]	.00	.00	.10	.25
Calculated content				
Crude protein	18.8	18.8	18.8	18.8
ME, kcal/kg	3318	3318	3315	3310
Ca, g/kg	.7	.7	.7	.7
P, g/kg	.6	.6	.6	.6
Chemical analysis				
Zn, mg/kg	91	118	86	111

[a]Supplied the following per kg diet; vitamin A, 4000 IU; vitamin D_3, 200 IU; vitamin E, 10 IU; riboflavin, 6 mg; niacin, 30 mg; d-pantothenic acid, 15 mg; vitamin B_{12}, 22 mcg; d-biotin, 1 mcg; folic acid, 4 mcg.

[b] When added to diet at .3% premixes provides the following in mg/kg: Cu, 5.; Fe, 94; Mn, 4.

[c] Formulated to provide 40 mg/kg Zn from ZnO when added to diet at .3%.

[d] Formulated to provide 100 mg/kg Zn from ZnO when added to diet at .3%.

[e]4% Zinc.

Table 1.2. Effect of diet on prechallenge performance of pigs

Item[a]	Zn oxide 40	Zn oxide 100	Zn met 40	Zn met 100	SE
Week 1					
ADG, kg	.41	.26	.45	.40	.05
ADFI, kg	.99	.73	.95	.98	.26
Gain:feed	.41	.31	.48	.40	.06
Plasma Zn, mg/dL	.73	.83	.81	.94	.06
Week 2					
ADG, kg	.36	.31	.29	.37	.05
ADFI, kg	1.09	1.00	1.05	1.12	.07
Gain:feed	.33	.30	.28	.33	.04
Plasma Zn, ppm[b]	.74	.85	.84	.97	.09
GSH Px units/mL	.85	.89	.90	.89	.11

[a]Abbreviation used: zinc methionine (Zn Met); Glutathione peroxidase (GSH Px) one unit was defined as the amount of enzyme that will convert 1 μmol NADPH to NADP per min at 20° C and pH 7.0. Number of pigs per group n = 4.

[b]Sources of zinc supplements differ, $P < .05$; Levels differ, $P < .03$; Level by source interaction, $P < .07$.

Table 1.3. Effect of diet on performance of pigs during the first week following challenge (third week of experiment)[a]

Item	Zn Oxide 40	Zn Oxide 100	Zn Met 40	Zn Met 100	SD
ADG, kg	-.04 (2)	-.02 (3)	.02 (3)	.00 (3)	.03
ADFI[b], kg	.48 (2)	.36 (3)	.75 (3)	1.03 (4)	.21
GSH Px,	1.5 (2)	1.8 (2)	1.6 (3)	1.7 (3)	.28
Plasma Zn, ppm[c]	.90 (2)	.96 (2)	1.33 (3)	1.04 (3)	.27

[a] Abbreviation used: zinc methionine (Zn Met); Glutathione peroxidase (GSH Px) one unit was defined as the amount of enzyme that will convert 1 μmol NADPH to NADP per min at 20° C and pH 7.0. Number of observations noted in parentheses.

[b] Sources of zinc supplement differ, $P < .02$. Source of supplement by level of supplement interaction, $P < .01$.

[c] Sources of zinc supplement differ $P < .1$. Source of supplement by level of supplement interaction, $P < .055$.

Table 1.4: Daily clinical scores of individual pigs following challenge with *S. hyodysenteriae*[a]

		DAY POST CHALLENGE																	
TRT	SEX	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	B	0	2	3	5	5	5	5	4	4	4	4	4	4	3	4	4	3	3	3
1	B	0	4	5	5	5	5	5	4	4	4	4	4	4	5	4	4	4	4	4
1	G	0	5	5	5	6														
1	B	0	2	5	6															
2	B	0	5	5	5	6														
2	B	0	1	1	2	5	5	5	4	0	0	0	0	0	0	0	0	0	0	0
2	B	4	4	5	5	5	5	5	5	5	5	6								
2	B	0	2	5	5	5	5	5	5	5	6									
3	G	1	1	2	4	5	5	4	4	4	3	4	3	4	1	3	4	3	2	2
3	G	0	1	1	1	1	1	4	5	5	5	5	5	2	5	5	4	5	5	5
3	G	0	0	2	5	5	5	3	3	1	3	2	3	0	0	0	0	0	0	0
3	B	0	5	5	5	6														
4	G	0	1	2	5	5	5	4	4	4	4	4	4	2	5	3	3	3	3	3
4	G	0	0	0	0	4	5	5	5	4	3	3	3	0	5	3	3	3	2	2
4	B	0	5	5	5	5	5	5	4	2	1	2	3	4	4	4	4	3	3	3
4	G	0	5	5	5	5	6													

[a]B = Barrow, G = Gilt. TRT = dietary treatment; 1 = 40 ppm zinc oxide, 2 = 100 ppm zinc oxide, 3 = 40 ppm zinc methionine, 4 = 100 ppm zinc methionine. Scoring system 0 = normal feces to 3 watery diarhea, additional point added each for blood or mucus in feces, score of 6 for animals found moribund or dead.

Table 1.5: Effect of dietary treatment on clinical scores for signs of swine dysentery during the first five days following challenge[a]

Day following challenge	Zn Oxide 40	Zn Oxide 100	Zn Met 40	Zn Met 100	SE
1	0	1.0	.25	0	.53
2	3.25	3.0	1.75	2.75	1.06
3[b]	4.5	4.0	2.5	3.0	.7
4[c]	5.25	4.25	3.75	3.75	.51
5	5.5	5.25	4.25	4.75	.51

[a] Scoring system 0 = normal feces to 3 watery diarhea, additional point added each for blood or mucus in feces, score of 6 for animals found moribund or dead.

[b] Level by source interaction, $P < .05$. Sources of zinc supplement differ, $P < .06$.

[c] Level by source interaction, $P < .06$. Sources of zinc supplement differ, $P < .08$.

PAPER 2: COMPARISON OF THE SOURCE OF DIETARY ZINC ON SELECTED IMMUNE FUNCTIONS AND CLINICAL SIGNS OF SWINE DYSENTERY IN YOUNG SWINE

ABSTRACT

Two studies were conducted to determine the effect of the source of supplemental zinc on selected immune functions of young pigs (Experiment 1), and the susceptibility of pigs to swine dysentery (Experiment 2). Thirty-six, 3 to 4 wk old pigs were fed one of three rations for 4 weeks during Experiment 1, then 34 of these pigs were continued on treatment for Experiment 2. Rations consisted of a corn soybean meal diet or the diet supplemented with 100 ppm Zn as ZnO or ZnMet. Pigs fed the basal diet had reduced proliferative response to conconavalin A ($P < .01$) and pokeweed mitogen ($P < .02$), after 2 wk of treatment, and a lower percentage of CD2 ($P < .02$), and CD8 ($P < .02$) after 4 wk compared with pigs fed supplemental Zn. In the second experiment, pigs fed supplemental Zn tended to have fewer signs of swine dysentery at 4 d ($P < .08$) and 5 d ($P < .07$) after challenge compared to pigs fed the basal diet. Pigs fed ZnMet consumed more feed than pigs fed ZnO ($P < .0001$), and pigs fed either supplement consumed more feed than pigs fed the basal diet ($P < .0001$). Under the conditions of these studies, the source of supplemental Zn did not have an effect on the immune function of young pigs or on their susceptibility to swine dysentery. Pigs fed ZnMet consumed more feed following challenge with *S. hyodysenteriae* than pigs fed ZnO.

Key words: Zinc, Swine Dysentery, Immune Function, Pig

INTRODUCTION

In mice, zinc deficiency results in rapid thymic atrophy and loss of T-helper function (Fraker et al., 1977), reduction in antibody production (Luecke et al., 1978), thymic hormone levels, Thy-1 positive lymphocytes (Bach et al., 1975), interleukin-1 and interleukin-4 production (Winchurch et al., 1987), natural killer cell activity (Fernandes et al., 1979), and phagocytic cell activity (Wirth et al., 1989). The thymic atrophy, depressed thymic hormone levels, and T-cell dysfunction observed in mice precedes the weight loss and acrodermatitis associated with zinc deficiency (Nash et al., 1979). Thus, a moderate zinc deficiency may result in impaired immune function, even though overt signs of zinc deficiency do not occur.

Administration of zinc has been shown to enhance survival of pigs following an intraperitoneal injection of *Salmonella pullorum.* (Miller et al., 1968). Pigs with enteric infections develop more severe signs of zinc deficiency than do uninfected pigs (Whitenack et al., 1978; Mansson, 1964). There are conflicting reports on the effects of zinc on survival of rats with bacterial infections. Zinc supplementation has been reported to enhance (Tocco-Bradley and Kluger, 1984) and reduce (Sobocinski et al., 1977) survival of rats infected with *Salmonella typhimurium.* The purpose of the studies reported here were to determine whether the source of zinc provided as zinc oxide (ZnO) or zinc methionine (Zn Met) has an effect on 1) selected immune functions or 2) the clinical signs of swine dysentery in young pigs.

MATERIALS AND METHODS

Experiment 1

Thirty-six, 3 to 4 wk old, crossbred pigs (Landrace X Yorkshire or Landrace X Yorkshire X Hampshire X Duroc) were grouped by litter and weight in a randomized block design. Treatments were assigned at random to pigs in each block. Pigs were housed individually in .3-m x .9-m expanded metal floored cages furnished with self-feeders and nipple waterers. Pigs were allowed ad libitum access to feed and water. Dietary treatments consisted of a corn, soybean meal, and dried whey basal diet, or the basal diet supplemented with a trace mineral premix formulated to provide an additional 100 ppm zinc (Table 2.1). Zinc was supplied in the premixes as either zinc oxide or zinc methionine. Feed intake and body weight were monitored weekly during the experiment.

Antibody response to KLH. Two groups (A and B) of 18 pigs each (six pigs per treatment) were injected with keyhole limpet hemocyanin (KLH). The pigs in group A were injected twice after 1 wk and again after 3 wk of dietary treatment. The KLH (.1 mg) was injected i. m. in the neck. The pigs in Group B were injected once with KLH, as previously described, 3 wk following the initiation of dietary treatment. Blood samples were collected from the orbital sinus on the day of each injection and 1 wk following each injection. Serum samples were stored at -20° C until ELISA tests for presence of antibody to KLH were completed. Serum antibody response to the KLH was determined using an ELISA assay. For this assay, KLH was diluted in coating buffer (.1 M $NaHCO_3$, pH 9.6) and 100 µL of the suspension was placed into each well of a 96 well microtiter plate (final concentration 2 µg/mL). The plate was incubated overnight at 4°C, the coating buffer was removed from the plate and the plates were either used for the ELISA assay immediately or stored at -20°C until the assay was completed. The plates were washed three times with Tween-Saline (TS, .85% NaCl, .025% Tween 20), then blocked for 1 h with 2% nonfat dry

milk in TS. Serum from each of the animals at each time point was diluted 1:50 in TS, and 100 μL was dispensed into the appropriate wells of the KLH coated microtiter plates. The plates were incubated at 37°C for 2 h. The plates were then washed as previously described. Goat antibody to swine immunoglobulin IgM (μ chain specific) conjugated to alkaline phosphatase was diluted to the appropriate dilution in TS, and 100 μL was dispensed into the appropriate wells of the microtiter plate. The plate was incubated as described for the swine sera. After washing, 200 μL of p-nitrophenyl phosphate substrate (Sigma Chemical Co.) was added to each well of the microtiter plate and allowed to incubate at 37°C for 20 to 60 min. The response was quantitated by measuring absorbance at 405 nm with an ELISA plate reader. The assay was repeated with a goat antibody specific for swine gamma chain conjugated to alkaline phosphatase (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD). Results were reported as mean optical density (OD) of three replicate wells.

Proliferative response. Heparinized whole blood was collected from pigs in group B for use in proliferation and lymphocyte subset assays. The ability of blood leukocytes to proliferate was evaluated using the mitogens conconavalin A (Con A, Sigma Chemical Co.), phytohemagglutinin (PHA, Burroughs Wellcome Diagnostics, Research Triangle Park, NC.), and pokeweed mitogen (PWM, Sigma Chemical Co.) using the following procedure. Heparinized whole blood was diluted 1 to 4 with RPMI 1640. The diluted blood (100 μL) was dispensed into the appropriate wells of U-bottom, sterile 96-well microtiter plates, and 100 μL of the diluted mitogen was then added to wells containing the diluted blood. Two levels of each mitogen were used for this study. The levels selected were based on preliminary studies conducted to develop a proliferation assay using whole blood, instead of gradient separated lymphocytes (data not shown). The two levels selected, for each mitogen (expressed as μg/mL final concentration) were: ConA 10, 5;

PHA 5, 1; PWM .1, .01. Plates were placed in a 37°C incubator with humidity, and 5% CO_2 for 5 d. Each well was then pulsed with ^{3}H-thymidine (.5 μCi/well, Amersham, Arlington Heights, IL) for 8 h. Cells were harvested onto glass microfiber filters using a Bellco cell harvester. Tritium incorporation was detected using a liquid scintillation spectrometer. Results were reported as mean counts per minute (cpm) of three replicate samples.

Lymphocyte subsets. Ten μL heparanized whole blood was pipetted into U-bottom, 96-well microtiter plates. Fifty μL of primary antibody (Table 2.2) were placed in each appropriate well, the plate was incubated on ice for 30 min. The wells were then washed three times with 150 μL of ice cold wash buffer (phosphate buffered saline, PBS, .05 mM EDTA, .1% NaN_3, and 1% fetal calf serum, FCS). Plates were centrifuged for 5 min at 200 x *g* between each wash. Fifty μL of a secondary antibody, goat anti-mouse conjugated to fluorescein isothiocyanate (FITC, Kirkegaard and Perry Laboratories, Inc.), was added to each well. The plate was incubated on ice for 30 minutes. Wells were washed twice as previously described. The cells were resuspended in 180 μL Coulter IMMUNO-LYSE™(Coulter Corp., Hialeah, FL.) working solution, with the addition of 20 μL of fixative within 30 s following lysis. Cells were washed once more, resuspended in wash buffer and stored at 4°C for up to 1 wk prior to analysis. Cells were analyzed with a Profile (Coulter Corp.) flow cytometer, using the 488 nm laser line from an argon ion laser. Indirect immunofluorescence from the FITC labeled antibodies was collected using a 515 long pass filter. Percentage of labeled cells were determined using the Profile (Coulter Corp.) or Reproman (Truefacts Software Inc., Seattle WA.) software.

Alkaline phosphatase activity was determined using a diagnostic kit (Sigma Chemical Co.) Leukocyte counts were determined using a Coulter Counter®, Coulter Corp., Hialeah, FL).

Data were analyzed by analysis of variance. The model included dietary treatment and block as main effects. Single df contrasts were made to compare basal and zinc supplemented treatments (basal vs ZnO + Zn Met), and source of zinc (ZnO vs Zn Met). Data are presented as least squares means. Statistical analyses were performed using the General Linear Model of SAS (1989).

Experiment 2

After the completion of the first experiment, 34 of the pigs were used immediately for experiment 2. For this experiment, pigs were housed in 6 rooms with 4 to 6 pigs per room, 2 rooms per treatment. Pigs from group A were penned together, by treatment group, as were pigs from group B. The rooms had concrete floors and were furnished with a self feeder and nipple waterer. Pigs were maintained on the same dietary source of zinc they had received in experiment 1. Feed and water were supplied ad libitum. Dietary treatments consisted of a corn and soybean meal grower diet (82 ppm Zn) or the grower diet supplemented with an additional 100 ppm zinc (Table 2.3). Additional zinc was supplied as trace mineral premixes with either ZnO or ZnMet as the sources of zinc. Feed intake was monitored as follows: 68 kg of feed were placed in each room's feeder, additional bags of feed were weighed and added to feeders as required. Pigs were weighed on the first and last day of the experiment.

Challenge. Pigs were experimentally infected on two consecutive days with 10^9 organisms per day. The organism used in this trial, *S. hyodysenteriae* strain B204, was grown as previously described (Nibbelink and Wannemuehler, 1991). Briefly, *S. hyodysenteriae* were grown anaerobically at 37°C in Trypticase Soy Broth (BBL Microbiological Systems, Cockeysville, MD.) supplemented with 5% horse serum (HyClone Laboratories, Logan, UT.) , .5% yeast extract (BBL Microbiological Systems),

and 1% VPI salt solutions (Solution A: $CaCl_2$, .4 g/L, and $MgSO_4$, .4 g/L; Solution B: $KHPO_4$, 2 g/L; KH_2PO_4, 2 g/L; $NaHCO_3$, 20 g/L; NaCl, 2 g/L). Log phase cultures were obtained. Prior to inoculation of the pigs, the numbers of bacteria were determined using a Petroff-Hauser counting chamber, and the bacteria were diluted to 10^7 organisms per mL with warm complete media.

Pigs were challenged twice, 24 h apart, by gastric intubation of a total volume of 100 mL culture broth (10^9 total organisms). Feed was removed the night before administration of the first dose of challenge innoculum, and feed was withheld until 4 h after the second administration of challenge innoculum. Feed was withheld a total of approximately 44 h. Pigs were monitored daily, after challenge, for clinical signs of disease. A clinical score of 0 to 5 was assigned to each pig daily. Scores were assigned for consistency of feces 0 = normal to 3 = watery diarrhea. An additional point each was added for blood or mucus present in feces. Daily scores of 2 to 5 were calculated by summing the scores for fecal consistency and the presence of mucus or blood. For example, to achieve a score of 5 a pig would have a waterey diarhea (3 points), plus blood (1 point) and mucus (1 point) in the feces. A score of 6 was assigned for pigs found moribund or dead. Nineteen days following challenge surviving pigs were weighed and necropsied. The thymus was removed and weighed. Blood samples were taken from the jugular vein for determining serum antibody response to *S. hyodysenteriae*. Cultures of cecal and large intestine contents were made and the presence of *S. hyodysenteriae* was verified by recovery of ß-hemolytic spirochetes on selective blood agar plates (Kunkle and Kinyon, 1986).

Antibody response. Serum antibody response to the *S. hyodysenteriae* whole cell lysate were determined using an ELISA assay. For this assay, lyophilized *S. hyodysenteriae* whole cell lysate was suspended in coating buffer (.1 M $NaHCO_3$, pH 9.6),

and 100 µL of the suspension was placed into each well of a 96 well microtiter plate. The plate was incubated overnight at 4°C, the coating buffer was removed from the plate, and the plates were either used for the ELISA assay immediately or stored at -20°C until the assay was completed. The plates were washed three times with Tween-Saline (TS, .85% NaCl -.025% Tween 20), then blocked for 1 h with 2% nonfat dry milk in TS. Serum from each of the animals at each time point was diluted 1:200 in TS and 100 µL was dispensed into the appropriate wells of the whole cell lysate coated microtiter plates. The plates were incubated at 37°C for 2 h. The plates were washed as previously described. Goat antibody to swine immunoglobulin (heavy and light chain specific) conjugated to alkaline phosphatase was diluted to the appropriate dilution in TS, and 100 µL was dispensed into the appropriate wells of the microtiter plate. The plate was incubated as described for the swine sera. After washing, 200 µL of p-nitrophenyl phosphate substrate (Sigma Chemical Co.) was added to each well of the microtiter plate and allowed to incubate at 37°C for 20 to 60 min. The response was quantitated by measuring absorbance at 405 nm with an ELISA plate reader. Results were reported as mean optical density (OD) of three replicate wells.

Data were analyzed by analysis of variance. The model included dietary treatment and block as main effects. Single df contrasts were made to compare basal and zinc supplemented treatments (basal vs ZnO + Zn Met), and source of zinc (ZnO vs Zn Met). Data are presented as least squares means. Statistical analyses were performed using the General Linear Model of SAS (1989).

RESULTS

Experiment 1

During weeks two and four, the pigs fed supplemental zinc consumed more feed ($P < .05$) and ADG was .2 to .3 kg greater ($P < .03$) than pigs fed the basal starter diet (Table 2.4). The pigs fed the basal diet also had a lower feed efficiency than pigs fed zinc supplemented diets during the second week ($P < .01$, Table 2.4). There were no significant differences among treatments during the first and third weeks of the experiment; although, there was a trend for the pigs fed the basal diet to consume less feed and gain less weight than the zinc supplemented pigs (Table 2.4). No clinical signs of parakeratosis were observed during the course of this experiment. One pig from the basal diet was removed from the study because of an inner ear infection.

There were no significant differences in antibody response to KLH injection (Table 2.5). There was a trend for pigs fe the basl diet to have a greater IgM response ($P < .056$), when the injection was given 1 wk following the initiation of dietary treatment. When the KLH injection was given after 3 wk of treatment there was a trend for the pigs on the basal diet to have a lesser IgM response than pigs fed supplemental zinc ($P < .058$).

There was no treatment effect on the leukocyte counts at any of the time points measured (Table 2.6). Prior to dietary treatment, there was a difference in the proliferative response to stimulation with PHA, at 5 μg/mL, between the pigs allotted to the ZnO treatment and those allotted to ZnMet treatment ($P < .02$). However, no differences were detected, prior to dietary treatment, between the basal and supplemented diets in the proliferative response to any of the mitogens used (Table 2.7). At the end of the second week of dietary treatment, the proliferative response to ConA (10 μg/mL, $P < .002$; 5 μg/mL, $P < .006$) and PWM (.1 μg/mL, $P < .02$; .01μg/mL, $P < .002$) was increased in the pigs fed the supplemental zinc compared with pigs fed the basal diet (Table 2.7). There

were no significant differences found between the basal and supplemented groups in the proliferative response to PHA (Table 2.7). A difference in the proliferative response to stimulation with PHA, at the 1 μg/mL level, was observed between the sources of zinc ($P < .02$). A difference in the response to ConA, at 10 μg/mL, was also observed between the two sources of zinc ($P < .02$). There were no significant differences in the proliferative response to any of the mitogens at the end of week 4 (Table 2.7). There was, however, a difference between the ZnO and ZnMet groups in the spontaneous proliferation rate ($P < .02$) as indicated by the RPMI control group (Table 2.7).

Analysis of the lymphocyte subsets revealed no significant differences among the treatment groups prior to initiation of dietary treatment or after two weeks of treatment (Table 2.8). There was a trend for the pigs fed zinc supplemented diets to have a higher CD4 to CD8 ratio ($P< .06$) at the end of the second week compared to pigs fed the basal diet. After 4 wk of dietary treatment, pigs fed the basal diet had a greater percentage of lymphocytes expressing CD2 or CD8 ($P < .02$) compared to pigs fed supplemental zinc (Table 2.8). The pigs on the basal diet also had a greater percentage of macrophages ($P < .02$) and tended to have a lower percentage of lymphocytes expressing class II ($P < .08$, Table 2.8).

Alkaline phosphatase levels were measured, after 4 wk of treatment, to determine if the basal diet was inducing a measurable zinc deficiency (data not shown). There was a trend for a reduction in the alkaline phosphatase activity of pigs fed the basal diet (322 IU/L) compared with pigs fed the zinc supplemented diets (468 IU/L for ZnO and 377 IU/L for ZnMet) but this was not significant.

Experiment 2

There was no significant diet effect on the clinical signs of swine dysentery. All of

the pigs exhibited clinical signs of disease after infection with *S. hyodysenteriae* strain B204. There was a trend for pigs fed supplemental zinc to have lower clinical scores on 4 d ($P < .083$) and 5 d after challenge ($P < .071$, Table 2.9). Pigs fed supplemental zinc consumed more feed than pigs fed the basal diet ($P < .0001$), and pigs fed ZnMet consumed more feed than pig fed ZnO ($P < .0001$, Table 2.10).

Three weeks after challenge, the surviving pigs were necropsied. Blood samples were collected for serum. The thymus was removed and weighed. Samples were taken from the cecum and large intestine to verify the presence of *S. hyodysenteriae*. The samples were examined by darkfield microscopy. All pigs were infected as determined by darkfield microscopy (data not shown). Pigs fed supplemental zinc as ZnMet tended to have larger thymuses than did the pigs fed the basal or ZnO diets (Table 2.10). There was no treatment effect on the antibody response to *S. hyodysenteriae* 3 wk following infection (Table 2.10).

DISCUSSION

Pigs fed the zinc supplemented diets had greater rate of gain and feed efficiency compared with pigs fed the basal diets for two of the four weeks monitored. Pigs fed the basal diet did not exhibit classic signs of zinc deficiency, such as parakeratosis, elevated leukocyte counts or lower serum alkaline phosphatase activity, compared with pigs fed zinc supplemented diets. Pigs fed either zinc oxide or zinc methionine supplements performed better than pigs fed the basal diet.

Prior to the initiation of dietary treatment, there was a difference in the proliferative response to PHA, at 5 μg per mL, between pigs allotted to the ZnO treatment and those allotted to the ZnMet treatment. Genetic differences in the proliferative response of porcine lymphocytes to ConA (Mallard et al., 1989, Edfors-Lilja et al., 1991) and PHA (Jensen and Christensen, 1980) have been previously described. Besides differences between litters, Edfors-Lilja et al. (1991) described large individual differences in the magnitude of proliferation to ConA from pigs within the same litter. It, therefore, seems likely that these individual differences in pigs are the explanation for the variability at the start of the experiment. This variation in an outbred population is to be expected; however, it does make interpretation of the data more difficult.

At the end of the second week of experiment 1, the pigs fed the supplemental zinc had higher proliferative responses to ConA and PWM compared with pigs fed the basal diet. There was no difference between the basal diet and the supplemented diet in the response to PHA stimulation. A difference in proliferative response to PHA, at 1 μg per mL, between pigs fed the two sources of zinc was observed. This difference was probably not due to treatment, because, a similar effect was observed prior to the initiation of treatment. By the end of 4 wk, no treatment effect was observed in the proliferative

response to any of the three mitogens. The transient changes in the effect of treatment on proliferative response may reflect a specific maturational effect of zinc on lymphocytes of pigs about 6 wk of age, or may indicate a change in the pigs requirement for zinc at this stage of development.

Differences in the lymphocyte subsets were found between pigs fed the basal diet and pigs fed the zinc supplemented diets. The pigs fed the basal diet had a higher percentage of cells expressing CD2 or CD8. These pigs also had a higher percentage of macrophages and tended to have a lower percentage of cells expression MHC class II antigen. This difference in lymphocyte subsets did not coincide with the observed differences in proliferative response to mitogens. The proliferative responses were observed after 2 wk of treatment, and the alterations in lymphocyte subsets were not observed until 4 wk of treatment. These changes in cell surface marker expression may be a reflection of a change in the maturational status of the pig. Pig lymphocytes are unusual in the pattern of cell surface antigens expressed compared with other species (Pescovitz et al, 1984,1985; Lunney and Pescovitz, 1987), and little is known about the functions of the different subsets. Zuckerman (1992) postulated that T cells of pigs that express both CD4 and CD8 are memory T cells. Because the reagents were not available to perform dual labeling analyses in this experiment, it is not known if the increase in CD8 lymphocytes was due to tincreases in cells expressing CD8 only or coexpressing CD4 and CD8. It is therefore, difficult to speculate on the significance of these changes in the lymphocyte subsets.

Although some differences in proliferation to mitogen and lymphocyte subsets were observed, the supplemental zinc did not have an effect on the antibody response of pigs to KLH. There was a trend for pigs fed the zinc supplemented diets to have a higher IgM response compared with pigs fed zinc supplemented diets when the injection was

given after 3 wk of treatment. However, there was also a trend for pigs fed the basal diet to have a greater IgM response compared with zinc supplemented pigs when the injection was given after 1 wk of dietary treatment. The observations made man, therefore, be due to differeces between the pigs used for the two sets of injections and not due to the dietary treatment.

Pigs fed supplemental zinc tended to have lower clinical scores on 4 d and 5 d after infection. Unlike the previous study discussed in this dissertation, there was no difference due to the source of zinc on clinical signs of disease. This may be due to the level of zinc selected for this study. During the course of infection with *S. hyodysenteriae*, pigs fed the supplemental zinc consumed more feed than pigs fed the basal diet. Pigs fed a diet supplemented with ZnMet consumed more feed than did pigs fed a diet supplemented with ZnO. There were no differences among treatments in the mortality rate. There was no difference among treatments in the antibody response of the pigs to *S. hyodysenteriae* 3 wk following infection. Pigs fed ZnMet tended to have a larger thymus, 3 wk after infection, compared with pigs fed either of the other diets. In cattle challenged with bovine rhinotracheitis virus, ZnMet enhanced recovery from infection (Chirase et al., 1991). Because this study was designed to investigate the effects of zinc on susceptibility to disease and not recovery, conclusions cannot be made about these effects in pigs. However, the increased feed intake and trend for pigs fed ZnMet to have larger thymuses may reflect a beneficial effect of this supplement with time on treatment. It seems plausible that pigs which remain on feed stand a better chance of recovering from a disease than those that have gone off feed.

IMPLICATIONS

It seems, from the trends observed in these two studies, that there may be a biological effect of zinc on immune function of young pigs. Under the conditions of these studies, no significant advantages could be attributed to either zinc oxide or zinc methionine as a source of supplemental zinc for young pigs.

LITERATURE CITED

Bach, J. F., M. Dardenne, J. M. Pleau, and M. Bach. 1975. Isolation, biochemical characteristics and biological activity of a circulation thymic hormone in the mouse and in the human. Ann. N. Y. Acad. Sci. 249: 186.

Chirase, N. K., D. P. Hutcheson, G. B. Thompson. 1991. Feed intake, rectal temperature, and serum mineral concentration of feedlot cattle fed zinc oxide or zinc methionine and challenged with infectious bovine rhinotracheitis virus. J. Anim. Sci. 69: 4137.

Edfors-Lilja, I., M. Bergstrom, U. Gustafsson, U. Magnusson, and C. Fossum. 1991. Genetic variation in Con A-induced production of interleukin 2 by porcine peripheral blood mononuclear cells. Vet. Immunol. Immunopathol. 27: 351.

Fernandes, G., M. Nair, K. Onoe, T. Tanaka, and R. Floyd. 1979. Impairment of cell-mediated immunity functions by dietary zinc deficiency in mice. Proc. Natl. Acad. Sci. USA 76: 457.

Fraker, P. J., S. M. Haas, and R. W. Luecke. 1977. Effect of zinc deficiency on the immune response of the adult A/J mouse. J. Nutr. 107: 1889.

Jensen, P. T., and K. Christensen. 1980. Genetic studies on the in vitro PHA transformation of porcine blood lymphocytes. Vet Immunol. Immunopathol. 2: 133.

Kunkle, R. A., and J. M. Kinyon. 1986. Selective medium for isolation of *Treponema hyodysenteriae* 67th Conference of Research Workers in Animal Diseases. Chicago, IL. (Abstr.).

Luecke, R. W., C. E. Simonel, and P. J. Fraker. 1978. The effect of restricted dietary intake on the antibody mediated response of the zinc deficient A/J mouse. J. Nutr. 108: 881.

Lunney, J. K., and M. D. Pescovitz. 1987. Phenotypic and functional characterization of pig lymphocyte populations. Vet. Immunol. Immunopathol. 17: 135.

Mallard, B. A., B. N. Wilkie, and B. W. Kennedy. 1989. Genetic selection for improved immune response in Yorkshire pigs. 2nd Int. Vet. Immunol. Symp. Hannover, 24-29 July. Immunobiol. Suppl. 4: 104. (Abstr.)

Mansson I. 1964. The intestinal flora in pigs with parakeratosis in swine. I. The intestinal flora with special reference to an atypical *Clostridium perfringens* and clinical observations. Acta Vet. Scand. 5: 279.

Miller, E. R., R. W. Luecke, D. E. Ullrey, B. V. Battzer, B. L. Bradley, and J. A. Hoeffer. 1968. Biochemical, skeletal, and allometric changes due to zinc deficiency in the baby pig. J. Nutr. 95: 278.

Nash, L., T. Iwata, G. Fernandes, R. A. Good, and G. Incefy. 1979. Effect of zinc deficiency on autologous rosette-forming cells. Cell. Immunol. 48: 238.

Nibbelink , S. K., and M. J. Wannemuehler. 1991. Susceptibility of inbred mouse strains to infection with *Serpula (Treponema)hyodysenteriae*. Infect. Immun. 59: 3111.

Pescovitz, M. D., J. K. Lunney, and D. H. Sachs. 1984. Preparation and characterization of monoclonal antibodies reactive with PBL. J. Immunol. 133: 368.

Pescovitz, M. D., J. K. Lunney, and D. H. Sachs. 1985. Murine anti-T4 and T8 monoclonal antibodies. Distribution and effects on proliferation and cytotoxic T cells. J. Immunol. 134: 37.

SAS. 1989. SAS/STAT® Users Guide: Statistics (Release 6.06) SAS Institute. Inc., Cary NC

Sobocinski, P. Z., W. J. Canterbury, and M. C. Powanda. 1977. Differential effect of parenteral zinc on the course of various bacterial infections. Proc. Soc. Exp. Biol. Med. 156: 334.

Tocco-Bradley, R., and M. J. Kluger. 1984. Zinc concentration and survival in rats infected with *Salmonella typhimurium*. Infect. Immun. 45: 332.

Whitenack, D. C., C. K. Whitehair, and E. R. Miller. 1978. Influence of enteric infection on zinc utilization and clinical signs and lesions of zinc deficiency in young swine. Amer. J. Vet. Res. 39: 1447.

Winchurch, R. A., J. Togo, and W. H. Adler. 1987. Supplemental zinc (Zn^{2+}) restores antibody formation in cultures of aged spleen cells II. Effects on mediator production. Eur. J. Immunol. 17: 127.

Wirth, J. J., P. J. Fraker, and F. Kierszenbaum. 1989. Zinc required for macrophage function: effect of zinc deficiency on uptake and killing of a protozoan parasite. J. Immunol. 68: 114.

Zuckerman, F. A., and R. J. Husman. 1992. Porcine CD4+CD8+ double positive lymphocytes represent primed T-cells and have a memory T-cell phenotype, $CD29^{bright}$ $LFA\text{-}1^{bright}$. 73rd Annual Meeting of the Conference of Research Workers in Animal Diseases, Chicago, IL. (Abstr.).

Table 2.1. Percentage composition of the basal starter diet, as fed

Ingredient	% of Diet
Ground corn	55.8
Soybean meal	28.7
Dried whey	10.0
Dicalcium phosphate	1.1
Calcium carbonate	.9
Vitamin-corn premix[a]	1.0
Mineral premix[b]	.3
Soybean oil	2.0
Antibiotic[c]	.175
Calculated analysis	
Crude protein, %	20.3
ME, kcal/kg	3361
Ca, g/kg	7.5
P, g/kg	6.1
Chemical analysis	
Zn, mg/kg[d]	67

[a]Supplies the following per kg diet; vitamin A, 4,000 IU; vitamin D_3, 200 IU; vitamin E, 10 IU; riboflavin, 6 mg; niacin, 30 mg; d-pantothenic acid, 15 mg; vitamin B_{12}, 22 mcg; d-biotin, 1 mcg; folic acid, 4 mcg.

[b]When added to diet at 0.3% premixes provides the following in mg/kg: Cu, 5.; Fe, 94; Mn, 4. For diets with supplemental zinc trace mineral premixes were formulated to provide Zn at 100 mg/kg. Zinc source either ZnO or zinc methionine.

[c]Supplied the following per kg of diet: santoquin, .44 mg; tiamulin, 39.6 mg.

[d]Chemical analysis of ZnO supplemented diet 186 ppm, ZnMet supplemented diet 168 ppm.

Table 2.2. Primary antibodies used for flow cytometric determination of lymphocyte subsets[a]

Antibody	Isotype	Specificity
MSA3	IgG 2b	SLA-DRw, MHC Class II
MSA4	IgG 2a	CD2, Pan T cell
HB147	IgG 2b	CD4
HB143	IgG 2a	CD8
HB142	IgG 2b	pig macrophage

[a]Antibody produced as cell culture supernates. Cell lines acquired from American Type Culture Collection

Table 2.3. Percentage composition of the basal grower diet, as fed

Ingredient	% of Diet
Ground corn	74.8
Soybean meal	19.9
Dicalcium phosphate	1.2
Calcium carbonate	.8
Vitamin-corn premix[a]	1.0
Mineral premix[b]	.3
Soybean oil	2.0
Calculated analysis	
Crude protein	16.0
ME, kcal/kg	3378
Ca, g/kg	.7
P, g/kg	.6
Chemical analysis	
Zn, mg/kg[c]	82

[a]Supplied the following per kg diet; vitamin A, 4,000 IU; vitamin D_3, 200 IU; vitamin E, 10 IU; riboflavin, 6 mg; niacin, 30 mg; d-pantothenic acid, 15 mg; vitamin B_{12}, 22 mcg; d-biotin, 1 mcg; folic acid, 4 mcg.

[b]When added to diet at 0.3% premixes provided the following in mg/kg: Cu, 5.; Fe, 94; Mn, 4. For diets with supplemental zinc trace mineral premixes formulated to provide Zn at 100 mg/kg. Zinc source either ZnO or zind methionine.

[c]Chemical analysis of ZnO supplemented diet 183 mg/kg; zinc methionine diet 183 mg/kg.

Table 2.4. Effect of dietary zinc supplement on pig performance[a]

Item	Basal	Zinc oxide	Zinc methionine	SE
Week 1				
ADFI, kg	.82[b]	1.07	.98	.07
ADG, kg	.51[cd]	.77	.72	.08
Gain:feed	.48	.71	.73	.10
Week 2				
ADFI, kg	.8[e]	1.14	1.07	.06
ADG, kg	.46[e]	.78	.75	.06
Gain:feed	.56[f]	.67	.69	.04
Week 3				
ADFI, kg	1.57	1.85[g]	1.84[g]	.15
ADG, kg	1.14	1.37	1.44	.10
Gain:feed	.73	.81	.83	.09
Week 4				
ADFI, kg	1.27[b](5)	1.76	1.72	.16
ADG, kg	.65[dh](5)	1.06	.89[d]	.10
Gain:feed	.45 (5)	.56	.44	.07

[a] Number of observations, n = 6, unless noted in parentheses.

[b]Basal differs from supplemented P < .04.

[c] Basal differs from supplemented P < .03.

[d]Some pigs had negative values for ADG.

[e]Basal differs from supplemented P < .001.

[f]Basal differs from supplemented P < .02.

[g]Some pigs had unusually low feed intake.

[h]Basal differs from supplemented P < .05

Table 2.5. Effect of dietary zinc supplement on antibody response to injection with KLH[a]

Item	Basal	Zinc oxide	Zinc methionine	SD
Primary IgM[b]	.193	.150	.112	.057
Secondary IgM[c]	.314 (5)	.256	.277	.081
Secondary IgG[c]	.521 (5)	.297	.514	.203
Primary IgM[d]	.218	.266	.293	.057

[a] Values expressed as mean optical density. Number of observations, n = 6, unless noted in parentheses.

[b]Injected pigs with .1mg KLH after 1 wk of dietary treatment. Response measured 2 wk after injection.

[c]Injected with .1 mg KLH a second time after 3 wk of dietary treatment. Response measured 1 wk following injection.

[d]Injected with .1 mg KLH after 3 weeks of dietary treatment. Response measured 1 wk following injection. Basal differs from supplemented, $P < .058$.

Table 2.6. Effect of dietary zinc supplement of total leukocyte count: expressed as millions of cells per mL

Time point	Basal	Zinc oxide	Zinc methionine	SE
Pretreatment	13.2	14.1	13.3	1.2
Day 14	22.8	22.8	20.4	2.3
Day 28	19.4	16.5	15.6	1.7

Table 2.7. Effect of dietary treatment on the proliferative response of peripheral blood cells to mitogens[a]

Mitogen, µg/mL	Basal	Zinc oxide	Zinc methionine	SD
Pretreatment				
None, RPMI only	821	880	692 (5)	270
ConA, 5	14,405	21,980	11,879 (5)	11,073
PHA, 5	161,196	204,445[b]	140,059[b] (5)	34,052
PHA, 1	36,330	37,166	35,804 (5)	24,220
PWM, .1	42,405	53,699	41,932 (5)	13,806
PWM, .01	7,703	6,945	5,342 (5)	4,322
Week 2,14 d				
None, RPMI only	844	822	944	530
ConA, 10	63,881[c]	117,758[b]	87,365[b]	17,841
ConA, 5	17,960[d]	55,204	35,805	15,940
PHA, 5	149,120	167,775	156,282	22,912
PHA, 1	43,829	82,098[b]	47,876[b]	21,193
PWM, .1	35,975[e]	55,580	44,667	10,192
PWM, .01	5,107[c]	10,836	9,263	2,291
Week 4, 28 d				
None, RPMI only	2,155	3,481[b]	1,575[b]	1,180
ConA, 10	51,002	74,735	50,492	25,075
ConA, 5	2,529	4,461	3,962	2,694
PHA, 5	151,282	167,581	245,881	162,518
PHA, 1	83,056	90,482	73,944	38,894
PWM, .1	38,348	41,221	36,911	13,171
PWM, .01	6,802	8,756	8,562	3,405

[a] Abbreviations used: conconavalin A, ConA; phytohemagglutinin, PHA; pokeweed mitogen, PWM. Values expressed as tritiated thymidine counts per minute. Number of observations, n = 6, except where noted in parentheses.

[b]Sources differ $P < .02$.

[c]Basal differs from supplemented $P < .002$.

[d]Basal differs from supplemented $P < .006$.

[e]Basal differs from supplemented $P < .02$.

Table 2.8. Effect of dietary zinc on the expression of lymphocyte cell surface markers[a].

Lymphocyte antigen	Basal	Zinc oxide	Zinc methionine	SD
Pretreatment				
MHC, Class II	32 (5)	33 (6)	30 (5)	5
CD2	48 (6)	41 (6)	45 (4)	9
CD4	18 (6)	21 (6)	22 (4)	4
CD8	27 (6)	27 (6)	25 (5)	6
MAC Ø	48 (5)	50 (6)	43 (5)	11
CD4/CD8 ratio	.7 (6)	.8 (6)	.9 (4)	.2
Week 2, 14 d				
MHC, Class II	35 (5)	38 (5)	39 (6)	9
CD2	56 (6)	58 (4)	55 (6)	9
CD4	24 (5)	28 (4)	30 (6)	6
CD8	39 (5)	34 (5)	38 (6)	7
MAC Ø	21 (5)	14 (3)	14 (5)	12
CD4/CD8 ratio	.6[b] (5)	.9 (4)	.8 (6)	.2
Week 4, 28 d				
MHC, Class II	39 (6)	56 (6)	53 (6)	15
CD2	63[c] (6)	50 (6)	53 (6)	8
CD4	35 (6)	30 (6)	34 (6)	11
CD8	44[b](6)	32 (6)	34 (6)	8
MAC Ø	25[d] (6)	10 (6)	13 (6)	9
CD4/CD8 ratio	.85 (6)	.95 (6)	1.06 (6)	.1

[a] Abbreviations used: MAC Ø = macrophage, MHC = major histocompatability complex, CD = cluster designation. Data presented as a percentage of lymphocytes as determined by flow cytometric analysis. Number of observations per group are designated by number in parentheses.

[b]Basal differs from supplemented $P < .06$.

[c] Basal differs from supplemented $P < .02$.

[cd]Basal differs from supplemented $P < .04$.

Table 2.9. Effect of dietary zinc on clinical signs of swine dysentery for the first five days after challenge with *Serpulina hyodysenteriae*[a]

Day after challenge	Basal	Zinc oxide	Zinc methionine	SE
1	.25	.9	.7	.6
2	1.2	1.3	1.0	.5
3	2.1	2.0	.9	.5
4	3.0[b]	2.5	2.5	.1
5	2.9[c]	2.5	2.5	.1

[a] Scores are mean score per pig per pen. Scoring system 0 = normal to 3 watery diarrhea, an additional point each for blood or mucus in feces. Moribund or dead animals score = 6.

[b] Basal differs from supplemented $P < .083$.

[c] Basal differs from supplemented $P < .071$

Table 2.10. Effect of dietary zinc supplement on pig performance, thymus size and antibody response to *Serpulina hyodysenteriae*

Item[a]	Basal	Zinc oxide	Zinc methionine	SE
Gain , kg	4.9	5.4	10.1	3
ADFI, kg	2.1[b]	2.4[c]	2.6[c]	.0
Thymus, g	15.1	16.8	57.6	9.6
Thymus, g/kg bw	.72	.83	1.78	.29
Antibody response[a]	.127	.110	.092	.028

[a]Gain refers to the average total weight gain of pigs surviving at 3 wk following challenge with *S. hyodysenteriae*, ADFI was calculated on the number of live pig days. Number of observation, n = 2.

[b]Basal differs from supplemented, P <.0001.

[c]Sources differ, P < .0001.

[d]Antibody response was measured 21 d after challenge with *S. hyodysenteriae*. Optical density, O.D., was calculated as the mean of 3 replicate wells for each live pig in each pen. Number of observations, n = 2.

PAPER 3: EFFECT OF ZINC DEFICIENCY AND ZINC SOURCE ON SELECTED IMMUNE FUNCTIONS IN YOUNG SWINE

ABSTRACT

Eighteen weanling, crossbred pigs were used to determine the effect of dietary zinc on selected immune functions of young swine. Pigs were fed a basal corn-soyprotein diet or the basal diet supplemented with trace mineral premixes formulated to provide 20 ppm Zn as either ZnO or zinc methionine (ZnMet). Pigs fed the basal diet exhibited clinical signs of Zn deficiency including dermatitis, reduced gain ($P < .04$) and gain to feed ratio ($P < .02$) compared with pigs fed the Zn supplemented diets. Zn deficiency of the pigs fed the basal diet was further confirmed by a lower concentration of serum zinc ($P < .02$), and reduced serum activity of the Zn dependent enzymes, alkaline phosphatase ($P < .0001$) and alanine amino transferase ($P < .03$). Hematocrit was elevated ($P < .04$) and lymphocytes, as a percentage of total leukocytes, ($P < .03$) were lower in pigs fed the basal diet. Pigs fed zinc supplemented rations had increased antibody responses to vaccination with *Serpulina hyodysenteriae* whole cell lysate compared to pigs fed the unsupplemented ration ($P < .01$). A greater proliferative response of blood leukocytes to pokeweed mitogen (.01 μg per mL) was observed in zinc supplemented pigs compared to pigs fed the basal diet ($P < .01$). After 45 d on treatment, the levels of bone ash as a percentage of dry matter, and the concentration of zinc in the bone ash was greater in zinc supplemented pigs compared with pigs fed the unsupplemented ration. At this time the concentration of zinc in serum ($P < .03$) and bone ash ($P < .05$) was greater in pigs fed ZnMet compared with pigs fed ZnO. Under the conditions of this study, immune function was enhanced by supplementing the basal diet with Zn. No differences in immune responses were observed between pigs fed the two sources of Zn. After 45 d of treatment pigs fed ZnMet had greater concentrations of zinc in serum and bone ash compared with pigs fed ZnO.

Key Words: Zinc, Immune Function, Pig

INTRODUCTION

The known effects of zinc on immune function have been reviewed recently (Keen and Gershwin, 1990). In mice, zinc deficiency results in rapid thymic atrophy and loss of T-helper function (Fraker et al., 1977), and reductions in antibody production (Luecke et al., 1978), thymic hormone levels, Thy-1 positive lymphocytes (Bach et al., 1975), interleukin (IL)-1 and IL-4 production (Winchurch et al., 1987), natural killer cell activity (Fernandes et al., 1979), and phagocytic cell activity (Wirth et al., 1989). The weight loss and acrodermatitis associated with zinc deficiency in mice is preceded by thymic atrophy, depressed thymic hormone levels, and T-cell dysfunction (Nash et al., 1979). Thus a moderate zinc deficiency may result in impaired immune function. Little is known about the effects of zinc on immune function in pigs. Zinc deficient pigs were observed by Miller et al. (1968) to have significantly reduced thymus weights and elevated leukocyte counts. The percentage of lymphocytes in these pigs was reduced and the immature neutrophil population was increased. The objectives of this experiment were to determine the effect of zinc on selected immune functions in young swine, and to compare the response to zinc supplemented as zinc oxide or zinc methionine.

MATERIALS AND METHODS

Eighteen, 3 to 4 wk old, crossbred pigs (Landrace X Yorkshire) were grouped by sex within a litter, in a randomized block design. Dietary treatments were randomly assigned to a pig in each block. All treatments were replicated six times: three replicates were barrows and the remaining three replicates were gilts. Average initial weight of the pigs was 7 kg. Pigs were housed individually in .3-m x .9-m expanded metal floored cages during the first 4 weeks of the experiment then moved to 1.2-m x 2.4-m raised deck pens for the remainder of the experiment. Each cage, or pen, was furnished with a self feeder and a nipple waterer. Pigs were allowed ad libitum access to feed and water. Dietary treatments consisted of a corn and isolated soyprotein basal diet or the basal diet supplemented with a trace mineral premix formulated to provide 20 ppm zinc (Table 2.1). Zinc, in the premix, was supplied as either zinc oxide (ZnO) or zinc methionine (ZnMet). The experimental period was 45 d. Pigs were weighed and feed intake was determined on days 7, 14, 21, 28, 39, and 45. The weight gain and feed intake were summed at the end of the experiment. The pigs were observed for signs of parakeratosis; no scores were assigned to the lesions. The criteria selected to evaluate immune function included antibody response to vaccination with *Serpulina hyodysenteriae* whole cell lysate and the proliferative response of peripheral blood leukocytes to the whole cell lysate or to the mitogens, phytohemagglutinin (PHA) or pokeweed mitogen (PWM).

To evaluate the antibody response the following vaccination protocol was used. Pigs were vaccinated i. m., three times with 1 mg per mL *S. hyodysenteriae* whole cell lysate using 50 percent Freund's incomplete adjuvant. One mL of vaccine was administered at each time point. The first vaccination was administered 14 d after the initiation of dietary treatment. The second vaccination was administered on day 28 and the third vaccination was

administered on day 39. Pigs were bled from the orbital sinus for serum at each vaccination and the last day of the experiment. In addition to serum, EDTA treated whole blood, and heparinzed whole blood were collected on 45 d for evaluation of proliferative response, hematologic response, and blood chemistry parameters. The pigs were then necropsied, and organ weights were obtained for spleen, liver, and thymus. The metatarsals of the left rear foot were collected from each animal for subsequent bone analysis.

Serum antibody response to the *S. hyodysenteriae* whole cell lysate was determined using an ELISA assay. For this assay, lyophilized *S. hyodysenteriae* whole cell lysate was suspended in coating buffer (.1 M NaHCO3, pH 9.6), to a final concentration of 50 μg/mL. A 100 μL aliquot of the suspension was placed into each well of a 96 well microtiter plate. The plate was incubated overnight at 4°C. The coating buffer was removed from the plate by shaking and the plates were either used immediately or stored at -20°C until the assay was completed. The plates were washed three times with Tween-Saline (TS; .85% NaCl, .025% Tween 20), and then blocked for 1 h with 2% nonfat dry milk in TS. Serum from each animal, at each time point, was diluted 1:200 in TS and 100 μL was dispensed into the appropriate wells of the whole cell lysate coated microtiter plates. The plates were incubated at 37°C for 2 h. The plates were washed as previously described. Goat antibody to swine immunoglobulin (heavy and light chain specific) conjugated to alkaline phosphatase (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) was diluted, and 100 μL was dispensed into the appropriate wells of the microtiter plate. The plate was incubated as described for the swine sera. The plates were washed, and 200 μL of p-nitrophenyl phosphate substrate (Sigma Chemical Co., St. Louis, MO.) was added to each well of the microtiter plate. The substrate was incubated at 37°C for 20 to 60 min. The assay was repeated with a goat antibody specific for swine gamma chain conjugated to alkaline

phosphatase (Kirkegaard and Perry Laboratories, Inc.). Results were reported as mean optical densities (OD) of three replicate wells.

To evaluate proliferative responses, peripheral blood was stimulated with *S. hyodysenteriae* whole cell lysate, PWM (Sigma Chemical Co.), or PHA (Burroughs Wellcome Co., Research Triangle Park, NC) . Heparinized whole blood was diluted 1 to 4 with RPMI 1640, and 100 µL of the diluted blood was dispensed into the appropriate wells of U-bottom sterile 96-well microtiter plates. A diluted mitogen (100 µL) was then added to each well containing the diluted blood. The mitogens were diluted in RPMI 1640 so that the following final concentrations (µg/mL) were achieved: whole cell lysate 1, 10, or 25; PWM .001, .01, or .1; PHA .5, 2.5, or 12.5. A range of mitogen concentrations were used because the optimal conditions for determining the differences between treatments were not known. Plates were placed in a 37°C incubator with humidity and 5% CO_2 for 5 d. Each well was pulsed with ^{3}H-thymidine (.5µCi/ well, Amersham, Arlington Heights, IL) for 8 h. Cells were harvested onto glass microfiber filters using a Bellco cell harvester. Tritium was detected using a liquid scintillation spectrometer. Results were reported as the stimulation index (SI) of three replicate samples. The SI was calculated according to the following formula: SI= mean cpm stimulated cells ÷ mean cpm of unstimulated cells.

Blood smears were prepared from the EDTA treated whole blood. EDTA whole blood, blood smears, and serum samples were analyzed for hematologic and blood chemistry parameters by Heartland Laboratories, Waterloo, Iowa.

Serum zinc levels were determined as previously described (Perry, 1990). Serum was diluted 1:4 in polypropylene tubes with a .03% solution of Brij 35 in distilled deionized water (ddH_2O). Zinc was then determined by flame atomic absorption spectrometry (Model 3100, Perkin Elmer, Norwalk, CT).

Bones were dissected out removing most of the muscle and soft tissue. The bones were boiled in ddH_2O for approximately 3 h, then scraped to remove remaining soft tissue. The bones were dried overnight at 100° C. Bones were placed in ceramic crucibles and ashed at 600°C in a muffle oven overnight. The percentage bone ash was determined by the equation: % ash = (g ash/g dry bone) X 100. The ashed bone was ground to a fine powder with a mortar and pestle. Bone ash (.5 g) was weighed and dissolved with 3 mL of 5 N HCl. This solution was diluted with ddH_2O to a final dilution of 1:250. Zinc was then determined by flame atomic absorption spectrometry.

Data were analyzed by analysis of variance. The model included dietary treatment, and block as main effects. The effect of sex and the treatment X sex interaction were also evaluated. Single df contrasts were made to compare basal and zinc supplemented treatments (basal vs ZnO and Zn Met) and the source of zinc (ZnO vs Zn Met). Statistical analyses were performed using the General Linear Model of SAS (1989). Data are presented as least squares means.

RESULTS

Performance and organ weight. Gain ($P < .04$) and gain to feed ratio ($P < .02$) were reduced for pigs fed the basal diet compared with the supplemented diets (Table 3.2). There was no significant difference in the performance of the pigs fed the ZnO supplemented diet vs pigs fed the Zn Met supplemented diet (Table 3.2). Five of the six pigs fed the basal diet exhibited clinical signs of parakeratosis. None of the pigs on the zinc supplemented diets developed signs of parakeratosis. No significant differences in organ size, standardized for body weight, were found among any of the treatment groups.

Blood chemistry. Pigs fed the basal diet had reduced concentrations of serum glucose ($P < .004$), and potassium ($P < .01$) whereas, total serum protein ($P < .03$) and, specifically, albumin ($P < .03$) were increased (Table 3.3). The activity of alkaline phosphatase (ALK) was 4 times lower for pigs fed the basal diet compared with pigs fed diets supplemented with either ZnO or ZnMet ($P < .0001$). Alanine amino transferase (ALT) activity was also lower for unsupplemented pigs ($P < .03$), and aspartate amino transferase (AST) was increased ($P < .01$) in the unsupplemented animals. There were no significant differences due to the source of zinc for any of the other blood chemistry parameters measured.

Hematology. Hematocrit (HCT, $P < .05$) was increased in pigs fed the basal diet compared with pigs fed supplemented diets (Table 3.4). Pigs fed the basal diet had 67 percent lymphocytes, as a percentage of total leukocytes, compared with 76 to 79 percent for pigs fed supplemental zinc ($P < .03$). There was a trend for pigs fed the basal diet to have an increased percentage of monocytes, and segmented and band neutrophils compared to pigs fed either zinc supplement, though this was not significant.

Immune function. Proliferative response to PHA, and *S. hyodysenteriae*, were not affected by dietary treatment (Table 3.5). The stimulation index for PWM at a concentration of .01 μg/mL was 3.5 times greater for pigs fed ZnO and, 4.8 times greater for pigs fed ZnMet than for pigs fed the basal diet, ($P < .01$, Table 3.5). No differences in stimulation index were found at the other levels of PWM used. Antibody responses to vaccination with *S. hyodysenteriae* also demonstrated a treatment effect on immune function (Table 3.6). Both the primary and secondary antibody response measured with the goat anti-swine, heavy and light chain specific, antisera was greater for pigs fed supplemental zinc, following the first ($P < .03$) and third vaccination ($P < .02$) than for pigs fed the basal diet. The gamma chain specific response was also greater after both the second and third vaccinations ($P < .04$ and .008, respectively) for pigs fed supplemental zinc. The source of zinc did not have an effect on the antibody response to *S. hyodysenteriae* whole cell lysate.

Serum zinc. Pigs fed the basal diet had serum concentrations of .32 ppm zinc after only 1 week on the diet compared to .6 ppm for supplemented pigs ($P < .0002$, Table 3.7). This reduced concentration of zinc persisted throughout the course of the experiment (Table 3.7). After 45 d on the diets, the zinc concentration in the serum was greater for pigs fed the ZnMet diet compared with pigs fed the zinc oxide supplemented diet (.59 vs .66 ppm, ZnO and ZnMet, respectively, $P < .03$).

Bone analysis. Bone ash for pigs fed the unsupplemented diet was 38% compared with 46% and 44% for pigs supplemented with ZnO or Zn Met, respectively ($P < .002$, Table 3.7). The concentration of zinc in bone ash for pigs fed the basal diet, was less than half that of supplemented pigs ($P < .0001$). Differences in the concentration of zinc in bone ash were also observed between the pigs fed ZnO (183.8 ppm) and pigs fed Zn Met (219 ppm, $P < .042$).

DISCUSSION

Pigs fed the basal diet exhibited classic clinical signs of zinc deficiency including parakeratosis, reduced gain, and gain to feed ratio. Serum alkaline phosphatase activity, and serum zinc concentrations were also reduced when compared with pigs fed diets supplemented with zinc. Many of the blood chemistry changes and hematologic changes are similar to those previously described for zinc deficient pigs (Miller et al., 1968). No changes were observed in the weight of the thymus (g/kg body weight) in this study. This conflicts with previous reports in pigs (Miller et al., 1968) and mice (Fraker et al, 1976).

The most sensitive indicator of zinc deficiency was serum alkaline phosphatase activities (Table 3.3). There was a four fold decrease in the activity of this enzyme in pigs fed the basal diet compared with pigs fed supplemental zinc. The activity of alanine amino transferase, another zinc dependent enzyme, was also reduced. Aspartate amino transferase activity was increased in the zinc deficient pigs. In previous studies with zinc deficient pigs, no changes in the activity of alanine or aspartate amino transferase were found (Burch et al., 1975). The decrease in blood glucose observed in the pigs on the basal diet is probably a manifestation of the reduced feed intake of pigs fed this diet.

The observed increase in total serum protein and albumin (Table 3.3), in the zinc deficient pigs, conflicts with previous reports (Miller, 1968). Usually, an increase in serum albumin is offset by an increase in blood volume and, therefore, not detected in blood chemistry tests. An exception to this would be in the case of dehydration caused by vomiting or diarrhea. One of the pigs on the basal diet was observed to have vomitting and diarrhea during the last 2 d of the experiment. It is possible that the other pigs on the basal diet were also vomitting or had loose stools but not observed doing so.

The level of potassium in zinc deficient pig sera was less than those of the supplemented pigs (Table 3.3), although both were within normal values (4.4-6.7) for pigs (Kaneko, 1973). There are several possible explanations for a reduction in the serum level of potassium for pigs fed the basal diet. The lower concentration may be the result of the reduced feed intake of pigs on the basal diet. A decrease in potassium levels would be consistent with the increase in total protein as an indication of dehydration in these pigs. There is also the possibility that the change is not a decrease in potassium levels for zinc deficient pigs, but may be an increase in the level of potassium for zinc supplemented pigs. This would be consistent with previous reports in which rats fed 1 mg of zinc per gram of diet increased plasma potassium levels above that of control rats receiving 37.5 μg of zinc per gram. There was no change in the plasma potassium in zinc deficient rats compared with the control rats (Song, 1987).

Miller et al. (1968) reported increased leukocyte counts with a decrease in the percentage of lymphocytes and an increase in the number of immature neutrophils in zinc deficient pigs. In this study, no difference was detected in the total leukocyte count between zinc deficient and zinc supplemented pigs. The data does support the previous observations (Miller et al., 1968) of decreases in the percentage of lymphocytes, and a trend toward more neutrophils with an increase in immature cells (Table 3.4).

Beneficial effects of zinc on the immune functions of pigs were indicated by the increased antibody response to vaccination with *S. hyodysenteriae*, and proliferative response to PWM in zinc supplemented pigs compared with unsupplemented pigs. The proliferative response to PHA or *S. hyodysenteriae* whole cell lysate was unaffected by dietary treatment. A difference in the effect of zinc on the proliferative response to PHA and PWM may be in the nature of the cells that these lectins stimulate. Phytohemagglutinin is classically considered a T-cell mitogen, and PWM is considered a T cell dependent B cell

mitogen. The lectins probably stimulate different T cell subsets. In mice, cells of the CD4+ T helper (T^H) subset have been divided into two functional groups based on the profile of cytokines produced: 1) T^H1 cells produce gamma-interferon and IL-2, giving rise to cell mediated immunity, 2) T^H2 cell cytokine secretion is predominately IL-4 and is responsible for antibody-mediated immunity (Mosmann and Coffman, 1989). Although these subsets have not yet been identified in the pig, the increase in the proliferation to PWM, and the increased antibody response to *S. hyodysenteriae*, may indicate that zinc has a beneficial effect on T^H2 cells. Other possible effects of zinc could be on the cell to cell interaction between either the T cells and the antigen presenting cells, between the T and B cells, or on B cells directly.

No differences in immune functions were found between the two groups of pigs receiving different types of zinc supplement. This may reflect the priority the body places on tissue distribution of zinc. Pigs fed the zinc methionine supplemented diet had higher concentrations of zinc in serum and bone after 45 d on the diet. This increase, in the body pool of zinc, could be of benefit during an infection when the acute phase response shifts zinc to liver, bone marrow, and thymus from other tissues.

Dietary zinc supplements resulted in improved antibody response to vaccination with *S. hyodysenteriae* whole cell lysate and proliferative response to PWM. Under the conditions of this experiment the source of zinc did not have an effect on immune function of young pigs.

IMPLICATIONS

The increase in proliferative response to pokeweed mitogen and in the antibody response to *Serpulina hyodysenteriae* whole cell lysate indicate that zinc is important for immune functions in the pig. The sources of zinc did not have an effect on immune functions under the conditions of this experiment. Zinc methionine may be more biologically available than zinc oxide as indicated by the higher levels of zinc in bone ash of pigs fed zinc methionine.

LITERATURE CITED

Bach, J. F., M. Dardenne, J. M. Pleau, and M. Bach. 1975. Isolation, biochemical characteristics and biological activity of a circulation thymic hormone in the mouse and in the human. Ann. N. Y. Acad. Sci.249: 186.

Burch, R. E., H. K. J. Hahn, and J. F. Sullivan. 1975. Serum and tissue enzymes activity and trace element content in response to zinc deficiency in the pig. Clin. Chem. 21: 568.

Fernandes, G., M. Nair, K. Onoe, T. Tanaka, and R. Floyd. 1979. Impairment of cell-mediated immunity functions by dietary zinc deficiency in mice. Proc. Natl. Acad. Sci. USA 76: 457.

Fraker, P. J., S. M. Haas, and R. W. Luecke. 1977. Effect of zinc deficiency on the immune response of the adult A/J mouse. J. Nutr. 107: 1889.

Kaneko, J. J. 1973. In: Standard Values in Domestic Animals, 3rd ed., Dept of Clinical Pathology, University of California, Davis.

Keen, C. L., and M. E. Gershwin. 1990. Zinc deficiency and immune function. Annu. Rev. Nutr. 10: 415.

Luecke, R. W., C. E. Simonel, and P. J. Fraker. 1978. The effect of restricted dietary intake on the antibody mediated response of the zinc deficient A/J mouse. J. Nutr. 108: 881.

Miller, E. R., R. W. Luecke, D. E. Ullrey, B. V. Battzer, B. L. Bradley and J. A. Hoeffer. 1968. Biochemical, skeletal, and allometric changes due to zinc deficiency in the baby pig. J. Nutr. 95: 278.

Mosmann, T., and R. L. Coffman. 1989. Heterogeneity of cytokine secretion patterns and functions fof helper T cells. Adv. Immunol. 46: 111

Nash, L., T. Iwata, G. Fernandes, R. A. Good, and G. Incefy. 1979. Effect of zinc deficiency on autologous rosette-forming cells. Cell. Immunol. 48: 238.

Perry, D. F. 1990. Flame atomic absorption spectrometric determination of serum zinc: collaborative study. J. Assoc. Off. Anal. Chem. 73(4): 619.

SAS. 1989. SAS/STAT® Users Guide: Statistics (Release 6.06) SAS Institute. Inc., Cary, NC.

Song, M. K. 1987. Influence of dietary zinc content on sodium and potassium metabolism in the rat. Mineral Electrolyte Metab. 13: 178.

Winchurch, R. A., J. Togo, and W. H. Adler. 1987. Supplemental zinc (Zn^{2+}) restores antibody formation in cultures of aged spleen cells II. Effects on mediator production. Eur. J. Immunol. 17: 127.

Wirth, J. J., P. J. Fraker, and F. Kierszenbaum. 1989. Zinc required for macrophage function: effect of zinc deficiency on uptake and killing of a protozoan parasite. J. Immunol. 68: 114.

Table 3.1. Percent composition of basal diet on an as fed basis

Ingredient	% of Diet
Ground corn	81.0
Isolated soy protein	12.4
Dicalcium phosphate	2.3
Calcium carbonate	.5
Vitamin-corn premix[a]	1.0
Mineral premix[bc]	.3
Soybean oil	2.0
Antibiotic[d]	.5
Calculated composition	
CP, %	18.1
ME, kcal/kg	3339
Ca, g/kg	7.0
P, g.kg	7.4
Chemical analysis	
Zn, mg/kg	32

[a]Supplies the following per kg diet; vitamin A, 4,000 IU; vitamin D_3, 200 IU; vitamin E, 10 IU; riboflavin, 6 mg; niacin, 30 mg; d-pantothenic acid, 15 mg; vitamin B_{12}, 22 mcg; d-biotin, 1 mcg; folic acid, 4 mcg.

[b]When added to diet at 0.3% premixes provides the following in mg/kg: Cu, 5.; Fe, 94; Mn, 4.

[c] Formulated to provide 20 mg/kg Zn when added to diet at 0.3%. Zinc was provided as ZnO or zinc methionine (ZnMet). Chemical analysis of supplemented diets: ZnO 59 mg/kg, ZnMet, 48 mg/kg).

[d]Supplied the following in mg/kg of diet: chlorotetracycline, 219.8; penicillin, 109.9; sulfamethazine, 219.8.

Table 3.2. Effect of zinc source on pig performance and organ size

Item[a]	Basal diet	Zinc Oxide	Zinc Methionine	SE
ADFI, kg	.72	.87	.94	.08
ADG, kg[b]	.32	.47	.48	.05
Gain : feed[c]	.43	.53	.52	.02
Thymus, g/kg BW	2.5	3.1	2.9	.26
Spleen, g/kg BW	2.3	2.2	1.9	.2
Liver, g/kg BW	24.8	26.0	25.3	1.3

[a]ADFI and ADG and gain: feed are calculated over the 45 d of the experiment. Organ weights were measured on the 45th d of the experiment. Number of observations, n = 6.

[b]Basal differs from supplemented, $P < .04$.

[c]Basal differs from supplemented, $P < .02$.

Table 3.3. Effect of zinc source on blood chemistry 45 days after initiation of dietary treatment

Item[a]	Basal diet	Zinc Oxide	Zinc Methionine	SE
BUN, mg/dL	15.7	14.3	14.5	.93
Creatinine, mg/dL	1.35	1.20	1.28	.05
BUN/Creatinine Ratio	12.0	12.1	11.5	.94
Osmolality	294	292	295	1.13
Total protein, g/dL	6.4	5.6	5.7	.2
Albumin, g/dL	3.5	3.2	3.2	.1
Gamma GT, IU/L	39.0	41.2	40.8	2.46
A/G Ratio	1.3	1.3	1.3	.11
Glucose, mg/dL	106[b]	121	120	2.95
Cholesterol, mg/dL	106	113	104	6.76
CPK, U/L	309	569	472	103
Amylase, IU/L	1,184	1,391	1,498	189
ALT, IU/L	21.2	30.3	26.2	2.3
ALK, IU/L	83[c]	351	360	20.2
LDH, IU/L	330	361	357	16.5
AST, IU/L	63.0	42.0	47.8	4.58
Na, mmol/L	147	146	147	.7
K. mmol/L	5.1	5.7	6.1	.2
Cl, mmol/L	106	88	107	10.3
Ca, mg/dL	10.4	10.6	10.6	.18
P, mg/dL	10.6	11.5	12.0	.57

[a]Abbreviations used: CPK = creatinine phosphokinase; BUN = blood urea nitrogen; A/G = albumin/globulin; ALT = alanine amino transferase; gamma GT = gamma globulin; ALK = alkaline phosphatase; LDH = lactate dehydrogenase; AST = aspartate aminotransferase. Number of observations, n = 6.

[b]Basal differs from supplemented, $P < .004$.

[c]Basal differs from supplemented, $P < .0001$.

Table 3.4. Effect of zinc source on hematological paramenters 45 days after initiation of dietary treatment

Item	Basal diet	Zinc oxide	Zinc methionine	SE
RBC, $10^6/mm^3$	9.2	8.4	8.7	.2
HGB, g/dL	14.4	13.9	14.1	.2
HCT[b], %	56[b]	51	53	1.3
MCV, μ^3	61	61	61	1.6
MCH, $\mu\mu g$	15.7	16.5	16.3	.5
MCHC, g/dL	25.8	27.2	26.6	.4
WBC, $10^3/mm^3$	21.2	18.7	19.5	1.7
SEG, %	28.2	17.7	21.2	3.6
BAND, %	.5	.0	.0	.2
LYMPH, %	67.3	79.5	76.3	3.3
MONO, %	1.5	.3	.3	.3
EOSIN, %	2.5	2.5	2.2	.6

[a]Abbreviations used: RBC = red blood cell; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; WBC = white blood cell; SEG = segmented neutrophils ; BAND = band neutrophils; LYMPH = lymphocytes; MONO = monocytes; EOSIN = eosinophils. Number of observations, n = 6.

[b]Basal differs from supplemented, $P < .05$.

Table 3.5. Effect of dietary zinc source on proliferative response of peripheral blood cells to mitogens and *S hyodysenteriae* antigen 45 days after initiation of dietary treatment

Item, µg/mL	Basal diet	Zinc Oxide	Zinc Methionine	SE
PHA, .5	42	58	65	22
PHA, 2.5	161	163	167	20
PHA,12.5	194	163	148	24
PWM, .001	1	2	1	.5
PWM, .01	8[b]	24	32	6
PWM, .1	147	165	189	30
S. HYO, 1	5	10	6	4
S. HYO, 10	8	15	12	6
S. HYO, 25	9	17	15	8

[a]Abbreviations used: PHA = phytohemagglutinin; PWM = pokeweed mitogen; S. HYO = *Serpulina hyodysenteriae* whole cell lysate. Values are expressed as a stimulation index. Mean counts per minute for unstimulated cultures = 1,154, no difference was detected between treatments in unstimulated cultures. Number of observations, n = 6.

[b]Basal differs from supplemented, $P < .01$.

Table 3.6. Effect of dietary zinc source on the porcine antibody response following vaccination with *S. hyodysenteriae*

Day[a]	Basal diet	Zinc oxide	Zinc methionine	SE
	O.D., Heavy and Light chain specific			
14	.056	.033	.022	.010
28	.374	.287	.397	.044
39	.729[c]	.878	.917	.048
45	.971	1.085	1.130	.054
	O.D., Gamma chain specific			
39	.411[b]	.560	.540	.040
45	.490[d]	.633	.643	.037

[a]Vaccinations were administered as follows: vaccination 1, 14 d; vaccination 2, 28 d, vaccination 3, 39 d. Response on 14 d represents a prevaccination level of antibody. Number of observations, n = 6.

[b]Basal differs from supplemented, $P < .04$.

[c]Basal differs from supplemented, $P < .02$.

[d]Basal differs from supplemented, $P < .01$.

Table 3.7. Effect of zinc source on concentration of zinc in serum and on bone ash and concentration of zinc in bone ash

Item[a]	Basal	Zinc Oxide	Zinc methionine	SE
Serum zinc, ppm				
14 d	.32[b]	.62	.59	.04
28 d	.29[c]	.67	.67	.03
39 d	.23	.49	.45	.06
45 d	.34[c]	.57[d]	.65[d]	.02
Bone Ash, % of dry matter	39[d]	46	45	1
Bone Zinc, ppm bone ash	100[c]	186[e]	221[e]	10

[a]Bone ash and bone zinc were determined 45 d after initiation of dietary treatment. Number of observations, n=6.

[b]Basal differs from supplemented, P< .0005.

[c]Basal differs from supplemented, P < .0001.

[d]Basal differs from supplemented, P < .02.

[e]Sources differ, P < .042.

PAPER 4: EVALUATION OF A MOUSE MODEL FOR DETERMINING THE EFFECT OF ZINC ON THE PATHOGENESIS OF SWINE DYSENTERY

ABSTRACT

These studies were designed to develop a mouse model to determine the effects of zinc on the pathogenicity of *Serpulina hyodysenteriae* infections. Mice fed a purified egg white and dextrose based diet were more susceptible to challenge with *S. hyodysenteriae* than had previously been observed with mice fed a conventional laboratory rodent chow diet. Mice (C3H/HeN) were fed a purified control (40 ppm) or low zinc (5 ppm) diet. All mice, regardless of diet, developed clinical signs of disease even with an infectious dose of 10^4 organisms per mouse. Mice (C3H/HeJ) fed the control zinc diet developed cecal lesions and were culture positive when infected with 10^2 organisms per mouse. A 50% infectious dose of $10^{3.7}$ was determined for BALB/cByJ mice fed the control zinc diet. The BALB/cByJ strain of mice, fed this purified egg white and dextrose diet may be a suitable model to study the effects of zinc or other factors which may effect the pathogenicity of *Serpulina hyodysenteriae*.

Key words: Zinc, *Serpulina hyodysenteriae*

INTRODUCTION

Swine dysentery is a mucohemorrhagic diarrheal disease of pigs caused by *Serpulina hyodysenteriae* (Taylor and Alexander, 1971; Glock and Harris, 1972). The pathogenesis of swine dysentery is attributed directly to enteric lesions because the organism does not invade beyond the lamina propria, and no lesions are found in other organs (Kinyon et al., 1980). The lesions are characterized histologically by erosion of the mucosal epithelium, inflammatory cell infiltration of the lamina propria, and coagulative necrosis of the superficial mucosa (Albassam et al., 1985; Hughes et al., 1975). Two virulence factors that may play a role in lesion development are a hemolysin (Saheb et al, 1980; Saheb and Lafluer, 1980; Lemcke and Burrows, 1982), and the lipopolysaccharide (LPS) (Baum and Joens, 1979; Nuesson et al., 1983). Other animal models used to study the pathogenesis of *S. hyodysenteriae* infections include chicks (Sueyoshi and Adachi, 1990), guinea pigs (Joens et al., 1978), and various strains of mice (Joens and Glock, 1979; Nibbelink and Wannemuehler, 1991; Nibbelink, 1992; Suenaga and Yamazaki, 1983). Mice infected with *S. hyodysenteriae* develop mucoid feces and cecal lesions similar to those found in pigs (Joens and Glock, 1979). However, clinical swine dysentery does not develop in mice.

There is conflicting evidence on the effects of zinc on resistance to bacterial infection. Zinc has been shown to enhance the survival of rats infected with *Salmonella typhimurium* (Tocco-Bradley and Kluger, 1984), *Francisella tularensis* , or *Streptococcus pneumoniae* (Sobocinski et al., 1977) and pigs infected with *Salmonella pullorum* (Miller et al., 1968). However, contrasting reports have indicated that zinc reduced survival in rats infected with *S. typhimuruim* or *Escherichia coli* (Sobocinski et al., 1977). The purpose

of these studies was to develop a mouse model to determine the effects of zinc on the ability of *S. hyodysenteriae* to infect and cause clinical signs of disease.

MATERIALS AND METHODS

General Protocol

Mice used in these trials were obtained from breeding colonies maintained at the Laboratory Animal Resource Facility of the College of Veterinary Medicine, Iowa State University, Ames, Iowa. The mice were maintained on Mouse Lab Chow #5010 (Purina Mills, Inc., St. Louis, MO) until the first day of the experiment when they were fed the experimental diets. The experimental diets used (Table 1) were Teklad Diet TD 85420 (Teklad, Madison WI), the control zinc diet (45 ppm), or Teklad Diet 89283, the low zinc diet (5 ppm). Mice had ad libitum access to feed and distilled water. The mice were placed on stainless steel wire racks in polypropylene cages to prevent the ingestion of additional zinc that may be present in the bedding materials.

Serpulina hyodysenteriae strain B204 was grown as previously described (Nibbelink and Wannemuehler, 1991). Briefly, *S. hyodysenteriae* were grown anaerobically at 37°C in trypticase soy broth (BBL Microbiological Systems, Cockeysville, MD) supplemented with 5% horse serum (Hyclone Laboratories, Logan, UT), .5% yeast extract (BBL Microbiological Systems), and 1% VPI salt solutions (Solution A: $CaCl_2$, .4 g/L, and $MgSO_4$, .4 g/L; Solution B: $KHPO_4$, 2 g/L; KH_2PO_4, 2 g/L; $NaHCO_3$, 20 g/L; NaCl, 2 g/L). Log phase cultures were used as the challenge inoculum. Prior to inoculation into mice, the numbers of bacteria were determined using a Petroff-Hauser counting chamber, and the bacteria were diluted to the appropriate concentration of organisms with warm complete media.

Mice were challenged by gastric intubation with 1 mL of culture broth, containing the appropriate concentration of organisms. The challenge material was administered twice, with 24 h between each dose. Feed was removed 6 h prior to the first dose of

challenge material and was returned 4 h after the second dose of challenge material. Feed was withheld a total of 34 hours. After challenge, the mice were allowed ad libitum access to the experimental diets until the time of necropsy.

Mice were euthanized at 10, 15, or 16 d following infection. The ceca were observed for macroscopic lesions. Scores were assigned to the cecal lesions as follows: no gross lesions (NGL) = 0; excess cecal mucus with no evidence of cecal atrophy = 1; cecal atrophy and excess mucus localized in the cecal apex only = 2; cecal organ atrophy and excess mucus = 3. Cecal atrophy was determined by comparison of the ceca from infected mice with ceca from uninfected mice. Other criteria used to indicate the presence of disease were isolation of *S. hyodysenteriae* from the cecal contents, and the number of colony forming units (CFU) per gram of cecal tissue.

S.erpulina hyodysenteriae was isolated by streaking cecal contents onto citrated-ovine blood agar plates containing antibiotics as described by Kunkle and Kinyon (1988). The plates were incubated anaerobically at 37°C for 96 h. Colonies with strong ß-hemolytic activity were examined by dark field microscopy to confirm them as large spirochetes.

To determine the number of CFU per gram of ceca, the ceca were removed aseptically and placed in sterile WhirlPak bags (Baxter Co., Minneapolis MN). The bags were weighed empty and reweighed after the ceca were placed in the bags. Cecal weight was determined by difference. After weighing, the ceca were suspended in 1:100 (w/v) phosphate-buffered saline (NaCl, 136.9 mM; Na_2PO_4, 8.1 mM; KH_2PO_4, 1.5mM; KCl, 2.7 mM; pH 7.2) and homogenized in a Stomacher Laboratory Blender (A. J. Stewart Co., London U.K.). A sample of the suspension was removed and examined by dark field microscopy for the presence of large spirochetes. Ten-fold serial dilutions were made of the suspension and the appropriate dilutions were added to 5 mL molten (45°C) Trypticase

Soy agar tubes supplemented with 5% ovine blood and antibiotics. The tubes were then vortexed and poured into 60 mM Petri dishes (#25010 Corning Glass Works, Corning N.Y.). The plates were incubated anaerobically at 37°C for 96 h. The zones of ß-hemolysis were enumerated and the CFU per g of cecal tissue were calculated.

Experiment 1

Thirty C3H/HeN mice were obtained from a breeding colony that were originally procured from Harlan Sprague Dawley, Indianapolis, Indiana. Fifteen mice were assigned to each of two dietary treatments: control zinc or low zinc (Table 4.1). The mice were housed in cages of one to five mice per cage. Fresh feed was given to each cage daily and any feed from the previous day that had not been consumed was removed. Feed intake was determined by difference. Feed intake was monitored during the first week of the experiment and again for the last 4 d of the experiment. On the 10th d of the experiment, feed was removed and the mice were infected with *S. hyodysenteriae*. Five mice from each diet treatment group were infected on two consecutive days with either 10^4, 10^5 or 10^6 organisms per mouse per day. Mice within a cage received the same infectious dose of organism. After the second infection the mice were allowed ad libitum access to feed. The mice were monitored daily for signs of diarrhea, or zinc deficiency. Sixteen days after infection the mice were euthanized. The ceca were removed, examined for gross clinical lesions, weighed, and cultured for *S. hyodysenteriae*.

Comparison of feed intakes between the two dietary treatments was made by analysis of variance using Statview II, Abacus Software Inc. A cage of mice served as the experimental unit. The mouse that died prior to infection was not used in the data analysis.

Experiment 2

Twenty-three C3H/HeJ mice, were obtained from a breeding colony originally procured from Jackson Laboratories, Bar Harbor, Maine. Mice were housed in four cages, with 5 to 7 mice per cage. Each cage of mice was assigned to an infectious dose of 10^5, 10^4, 10^3, or 10^2 organisms per mouse. The mice were fed the Teklad Diet TD 85420, control zinc, for 14 d prior to infection with *S. hyodysenteriae*. Feed was removed and the mice were infected on two consecutive days with the appropriate number of bacteria. Ten days following infection the mice were euthanized and the ceca were examined for the presence of gross clinical signs of infection. Cultures of the cecal contents were made to detect the presence of spirochetes.

Experiment 3

Twenty-eight BALB/cByJ mice were obtained from a breeding colony originally procured from Jackson Laboratories, Bar Harbor, Maine. The mice were caged with 4 to 6 mice per cage. Each cage of mice were assigned to an infectious dose of 10^6, 10^5, 10^4, 10^3, or 10^2 organisms. The mice were allowed ad libitum access to the Teklad Diet TD 85420, control zinc, for 10 d prior to infection with *S. hyodysenteriae*. Fifteen days after infection the mice were euthanized. The ceca were examined for the presence of gross clinical signs of infection, and cecal contents were examined by darkfield microscopy for the presence of spirochetes. A fifty percent infectious dose was calculated using the method of Reed and Muench (1938).

RESULTS

Experiment 1

The mean feed intake of the low zinc group for the first week prior to infection was significantly greater ($P < .01$) than that of the control zinc group. After infection, there was no difference in the feed intake between the two groups (Table 4.2). There was no significant difference between the initial weight of the mice, and there were no significant differences in the amount of weight gained between the groups (data not shown).

One mouse died on the third day of the experiment. This death was prior to infection and was probably due to hypothermia. All of the mice exhibited signs of stress, such as rough coat, at this time. A heat lamp was placed above the cages and the condition of the mice improved quickly. A second mouse died during the course of the infection.

All infected mice had clinical signs of disease (Table 4.3). There was no difference in the number of organisms isolated per gram of ceca due to dietary zinc level.

Experiment 2

In this experiment all mice exhibited gross cecal lesions (Table 4.4), and were culture positive for *S. hyodysenteriae* (data not shown) even at an infectious dose as low as 10^2.

Experiment 3

Mice, BALB/cByJ, challenged with 10^4, 10^5, or 10^6 organisms developed cecal lesions by 10 days following inoculation. Three of four mice challenged with 10^3 organisms had cecal lesions and one of six mice challenged with 10^2 organisms developed cecal lesions (Table 4.5). The severity of the lesions, as reflected by total cecal scores, decreased in a dose dependent manner from 10^6 to 10^3 infectious organisms (Table 4.5). A fifty percent infectious dose (ID_{50}) of $10^{3.7}$ organisms was calculated using the method of Reed and Muench (1938).

DISCUSSION

It is evident from these three experiments that there is an enhancing effect of the Teklad diet on *S. hyodysenteriae* infections. This effect was observed with all three strains of mice studied. Further descriptions of the use of this diet, as an enhanced model of *S. hyodysenteriae* infection, in mice were given by Nibbelink and Wannemuehler (1992). The egg white based diet seems to have an effect that results in a decrease in the ID_{50} for the C3H/HeN, C3H/HeJ, and the BALB/cByJ mice from the 10^6, or more, organisms previously determined by Nibbelink and Wannemuehler (1991) for mice fed mouse lab chow. The ID_{50} of $10^{3.7}$ represents a dramatic increase in susceptibility for the BALB/cByJ mice, previously reported to be refractory to infection when fed normal mouse chow (Nibbelink and Wannemuehler, 1991). The reason for the increased susceptibility to *S. hyodysenteriae*, of mice fed the Teklad diet is unclear. Nibbelink and Wannemuehler (1992) reported a 4 log increase in the number of gram positive organisms recovered from the cecum and a 2 log increase in the number of gram negative organisms. This may indicate a shift in the normal cecal microflora or a change in the availability of growth substrates that would facilitate colonization of the ceca by *S. hyodysenteriae* or other organisms. Meyer et al. (1974a,b) found that some bacteria can serve as synergistic organisms for *S. hyodysenteriae*, Therefore, a shift in the gut microflora may have an impact on the outcome of disease if there were an increase in these synergistic bacteria.

Although both the Teklad Control and the Mouse Chow diets are complete diets, they are very different in composition and nutrient content. Multiple factors could, and probably do, contribute to the increased susceptibility of mice to *S. hyodysenteriae*. Of interest to this investigation is the difference in total zinc content of the diet. Zinc analysis of the two feeds provided by Purina and Teklad guarantee that the mouse chow diet

provides 124 ppm zinc while the Teklad Control diet provides 46 ppm zinc from zinc carbonate. Mice have previously been shown to maintain normal growth and normal antibody response when fed a diet containing zinc at 5.9 mg/kg (Luecke and Fraker, 1979). The level of zinc in either the Teklad or the Mouse Chow diets should be adequate for the mice to mount normal immune responses to *S. hyodysenteriae*. That the Teklad diet is sufficient for a normal immune response is supported by equivalent antibody responses to injection with *S. hyodysenteriae* whole cell lysate, when fed either Teklad or Mouse Chow diets (Nibbelink and Wannemuehler, 1992). If the zinc content of the diet is involved in the increased susceptibility of mice to *S. hyodysenteriae*, it apparently is not due to an effect on the acquired immune responses of the mice.

IMPLICATIONS

The susceptibility to infection is greatly increased when C3H/HeN, C3H/HeJ, and the BALB/cByJ mice are fed the Teklad control zinc diet compared to normal mouse chow. Since experiment 1 did not demonstrate any differences between the control and low zinc Teklad diets, a model using the BALB/cByJ mouse and the ID_{50} of $10^{3.7}$ for *S. hyodysenteriae* infection, may be useful in trying to determine the effects of zinc on *S. hyodysenteriae* infection.

LITERATURE CITED

Albassam, M. A., H. J. Olander, H. L. Thacker, and J. J. Tureck. 1985. Ultrastructural characterization of colonic lesions in pigs inoculated with *Treponema hyodysenteriae*. Can. J. Comp. Med. 49: 384.

Baum, D. H., and L. A. Joens. 1979. Serotypes of beta-hemolytic *Treponema hyodysenteriae*. Infect. Immun. 25: 792.

Glock, R. D., and D. L. Harris. 1972. Swine dysentery II: Characterization of lesions in pigs inoculated with *Treponema hyodysenteriae* in pure and mixed culture. Vet. Med. Small Anim. Clin. 67: 65.

Hughes, R., H. J. Olander, and C. B. Williams. 1975. Swine dysentery: pathogenicity of *Treponema hyodysenteriae*. Am. J. Vet. Res. 36: 971.

Joens, L. A., J. G. Songer, D. L. Harris and R. D. Glock. 1978. Experimental infection with *Treponema hyodysenteriae* in guinea pigs. Infect. Immun. 22: 132.

Joens, L. A., and R. D. Glock. 1979. Experimental infection in mice with *Treponema hyodysenteriae* Infect. Immun. 25: 757

Kinyon, J. M., D. L. Harris, and R. D. Glock. 1980. Isolation of *Treponema hyodysenteriae* from experimentally infected pigs at various intervals post inoculation. Proc. Int. Congr. Pig Vet. Soc., Copenhagen.

Kunkle, R. A., and J. M. Kinyon. 1988. Improved selective medium for the isolation of *Treponema hyodysenteriae*. J. Clin. Microbiol. 26: 2357.

Lemcke, R. M., and M. R. Burrows. 1982. Studies on a haemolysin produced by *Treponema hyodysenteriae*. J. Med. Microbiol. 15: 205.

Luecke, R. W., and P. J. Fraker. 1979. The effect of varying dietary zinc levels on growth and antibody-mediated response in two strains of mice. J. Nutr. 109: 1373.

Meyer, R. C., J. Simon, and C. S. Byerly. 1974a. The etiology of swine dysentery. I. Oral inoculation of germ-free swine with *Treponema hyodysenteriae* and *Vibrio coli*. Vet. Path. 11: 515.

Meyer, R. C., J. Simon, and C. S. Byerly. 1974b. The etiology of swine dysentery II. Effect of a known microbial flora, weaning and diet on disease production in gnotobiotic and conventional swine. Vet. Path. 11: 527.

Miller, E. R., R. W. Luecke, D. E. Ullrey, B. V. Battzer, B. L. Bradley, and J. A. Hoeffer. 1968. Biochemical, skeletal, and allometric changes due to zinc deficiency in the baby pig. J. Nutr. 95: 278.

Nibbelink, S. K. 1992. The role of tumor necrosis factor and secondary bacteria in the pathogenesis of swine dysentery. Ph. D. Dissertation. Iowa State University, Ames, IA.

Nibbelink , S. K., and M. J. Wannemuehler. 1991. Susceptibility of inbred mouse strains to infection with *Serpula (Treponema) hyodysenteriae*. Infect. Immun. 59: 3111.

Nibbelink, S. K., and M. J. Wannemuehler. 1992. An enhanced murine model for studies on the pathogenesis of *Serpulina hyodysenteriae*. Infect. Immun. 60: 3433.

Nuessen, M. E., L. A. Joens, and R. D. Glock. 1983. Involvement of lipopolysaccharide in the pathogenicity of *Treponema hyodysenteriae*. J. Immunol. 131: 997.

Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty per cent endpoints. Am. J. Hygiene 27: 493.

Saheb, S. A., and L. Lafleur. 1980. Characteristics of the interaction of a treponemal hemolysin with rabbit erythrocytes. Biochimie 62: 787.

Saheb, S. A., L. Massiocotte, and B. Picard. 1980. Purification and characterization of *Treponema hyodysenteriae* hemolysin. Biochimie 62: 779.

Sobocinski, P. Z., W. J. Canterbury, and M. C. Powanda. 1977. Differential effect of parenteral zinc on the course of various bacterial infections. Proc. Soc. Exp. Biol. Med. 156: 334.

Suenaga, I., and T. Yamazaki. 1983. Experimental infection with *Treponema hyodysenteriae* in nude mice. Zbl. Bakt. Hyg. I Abt. Org. A 254: 528.

Sueyoshi, M., and Y. Adachi. 1990. Diarrhea induced by Treponema *hyodysenteriae*: a young chick cecal model for swine dysentery. Infect. Immun. 58: 3348.

Taylor, D. J., and T. J. L. Alexander. 1971. The production of dysentery in swine by feeding cultures containing a spirochete. Br. Vet. J. 127: viii.

Tocco-Bradley, R., and M. J. Kluger. 1984. Zinc concentration and survival in rats infected with *Salmonella typhimurium*. Infect. Immun. 45: 332.

Table 4.1. Composition of the Control and Low Zinc Diets

Ingredient	Low Zinc, g/kg	Control Zinc, g/kg
Egg white solids, spray dried	200.0	200.0
Dextrose, monohydrate	634.2658	634.2658
Corn oil	100.0	100.0
Cellulose (fiber)	29.9904	29.9107
Zinc carbonate, $ZnCO_3$[b]	.0096	.0893
Calcium phosphate, dibasic, $CaHPO_4$	19.767	19.767
Magnesium sulfate, $MgSO_4$	2.4752	2.4752
Potassium chloride, KCl	2.2882	2.2882
Sodium chloride, NaCl	.7781	.7781
Ferrous sulfate, $FeSO_4 \cdot 7H_2O$	.2	.2
Manganese sulfate, $MnSO_4 \cdot H_2O$	.1662	.1662
Cupric sulfate, $CuSO_4$	.0151	.0151
Potassium iodate, KIO_3	.0004	.0004
Chromium potassium sulfate, $CrK(SO_4)_2 \cdot 12H_2O$	.02	.02
Vitamin mix, Teklad (#40060)[a]	10.0	10.0
Biotin	.004	.004
Ethoxyquin (antioxidant)	.02	.02

[a] Vitamin mix supplies the following grams per kilogram of diet: p-aminobenzoic acid, .11; ascorbic acid, .99; d-biotin, .0004; vitamin B_{12}, .00003; d-calcium pantothenate, .066; choline, 1.43; folic acid, .002; inositol, .11; menadione, .05; niacin, .099; pyridoxine•HCl, .022; riboflavin, .022; thiamin•HCl, .022. The following are supplied in units/kg: vitamin A, 19,824; vitamin D_3 2,202.5; vitamin E 121.15.

[b] Low Zinc diet provides at least 5 ppm zinc and control zinc diet will provide 46 ppm zinc from zinc carbonate.

Table 4.2. Effect of dietary zinc on feed intake of C3H/HeN mice during the preinfection and post infection periods

Diet	Number of mice per cage	ADFI, g[a] Pre infection	ADFI, g[a] Post infection
control	5	2.9	3.2
control	5	3.0	3.0
control	5	2.8	2.5
	AVG.	2.9[b]	2.9
low zinc	5	3.2	2.0
low zinc	5	3.1	3.6
low zinc	2	3.3	3.2
low zinc	1	[c]	[c]
low zinc	2	3.3	3.0
	AVG.	3.2[b]	3.0
	SE	.05	.3

[a]ADFI is expressed as mean per mouse.

[b]Different from each other, $P < .01$.

[c]Mouse died prior to infection.

Table 4.3. Effect of dietary zinc on the colonization of the ceca of C3H/HeN mice by *S. hyodysenteriae*

Diet	Number of mice	Infectious dose[a]	Cecal weight, g	Organisms, millions per g ceca
control	5	10^4	.104	12.1
control	5	10^5	.102	19.6
control	5	10^6	.112	8.9
low zinc	5	10^4	.122	8.5
low zinc	5	10^5	.085	29.9
low zinc	4	10^6	.123	4.1

[a]Number of organisms per mL inoculum.

Table 4.4. Gross cecal lesions of C3H/HeJ mice infected with *S. hyodysenteriae* fed the Teklad control diet

Infectious dose[a]	Number of mice	Total Score[b]	Score per mouse[c]
10^5	7	21	3.00
10^4	5	9	1.80
10^3	5	9	1.80
10^2	6	16	2.67

[a]Number of organisms per mL inoculum.

[b]Sum of lesions scores for the group of mice. Scores were as follows: no gross lesions = 0; excess cecal mucus with no evidence of cecal atrophy = 1; cecal atrophy and excess mucus localized in the cecal apex only = 2; cecal organ atrophy and excess mucus = 3.

[c]Mean score per mouse.

Table 4.5. Gross cecal lesion scores of BALB/cByJ mice fed the Teklad control diet and infected with *S. hyodysenteriae*

Infectious dose[a]	Mice with lesions	Total mice	Total score[b]	Score per mouse[c]
10^6	5	6	15	2.50
10^5	5	6	14	2.33
10^4	5	6	9	1.50
10^3	3	4	3	.75
10^2	1	6	3	.50

[a]Number of organisms per mL inoculum.

[b]Sum of lesions scores for the group of mice. Scores were as follows: no gross lesions = 0; excess cecal mucus with no evidence of cecal atrophy = 1; cecal atrophy and excess mucus localized in the cecal apex only = 2; cecal organ atrophy and excess mucus = 3.

[c]Mean score per mouse.

PAPER 5: EFFECT OF THE LEVEL OF SUPPLEMENTAL ZINC IN DRINKING WATER OF MICE ON THEIR SUSCEPTIBILITY TO SERPULINA HYODYSENTERIAE INFECTION

ABSTRACT

The effect of zinc on the susceptibility of BALB/cByJ mice to *Serpulina hyodysenteriae* infection was investigated using four levels of zinc sulfate in the drinking water of the mice. Mice were maintained on a purified egg white and dextrose diet containing 46 ppm zinc from zinc carbonate. Water was supplemented with zinc sulfate heptahydrate to provide 12.5, 25, 50, or 100 mg/dL. Mice were infected with 10^4 organisms per mouse. The mice were euthanized 3 d or 10 d following infection and examined grossly and histologically for the presence of cecal lesions. No gross signs of disease were observed in any mice 3 d following infection. Ten days following infection, mice receiving 12.5 ppm zinc in the water had fewer cecal lesions ($P < .05$) than mice receiving no supplemental zinc or 50 ppm zinc. Mice receiving water with 25 ppm zinc had scores intermediate between 12.5 and 50 ppm. On histologic examination, mice receiving no supplemental zinc or 12.5 ppm supplemental zinc had lymphoplasmacytic cell infiltration into the lamina propria whereas mice receiving 25, 50, or 100 ppm of zinc had a mixture of neutrophilic and lymphoplasmacytic cell infiltration. At 12.5 ppm, zinc supplementation in the drinking water reduced the gross cecal lesions observed in BALB/cByJ mice. The effect of zinc supplementation in the drinking water of mice was dose dependent and had a narrow range of effectiveness.

Key words: Zinc, *Serpulina hyodysenteriae*, Infectious Disease

INTRODUCTION

Serpulina hyodysenteriae is the causative agent of swine dysentery, a mucohemorrhagic diarrheal disease of pigs (Taylor and Alexander, 1971; Glock and Harris, 1972). The lesions associated with this disease are characterized by erosion of the mucosal epithelium, inflammatory cell infiltration of the lamina propria, and coagulative necrosis of the superficial mucosa (Albassam et al., 1985; Hughes et al., 1975). Previous studies, at Iowa State University, suggested that zinc supplementation of swine diets with zinc methionine reduced the severity of clinical signs of swine dysentery (Wannemuehler and Crump, personal communication). Two virulence factors that may play a role in lesion development are a hemolysin (Saheb et al., 1980; Saheb and Lafleur, 1980; Lemcke and Burrows, 1982), and the lipopolysaccharide (LPS) (Baum and Joens, 1979; Nuessen et al. 1983; Nibbelink and Wannemuehler, 1991). The hemolytic activity of the ß-hemolysin on sheep red blood cells has been reported to be inhibited *in vitro* by 5 mM zinc or copper (Hyatt et al., 1992). The effects of zinc on the activity the hemolysin *in vivo* are not known. Administration of zinc to mice has been shown to be protective against challenge with *Salmonella typhosa* endotoxin (Snyder and Walker, 1976) or *Salmonella typhimurium* endotoxin (Sobocinski et al., 1977b). Protection against an i.p. injection of *Salmonella typhimurium* endotoxin was effective only when zinc was also administered i.p., but zinc was not protective when administered i.v. (Sobocinski et al., 1977b). Previous studies have shown that mice fed Teklad egg white based diets are more susceptible to infection with *S. hyodysenteriae* than are mice fed the normal mouse chow diet (Nibbelink and Wannemuehler, 1992). These diets differ in many ways, including the level of zinc in the diets. The normal mouse chow diet contains two to three times the zinc (124 ppm) as the Teklad control diet (46 ppm). Therefore, zinc may be a factor in the prevention of lesion

development in mice infected with *S. hyodysenteriae*. The purpose of this investigation was to determine if increasing zinc at levels fed mice above that found in the control Teklad diet would effect the susceptibility of the mice to infection with *S. hyodysenteriae*.

MATERIALS AND METHODS

Mice used in these trials were obtained from breeding colonies maintained at the Laboratory Animal Resource Facility of the College of Veterinary Medicine, Iowa State University, Ames, Iowa. The mice were maintained on Mouse Lab Chow #5010 (Purina Mills, Inc., St. Louis, MO) until the first day of the experiment when they were fed the experimental diet. Sixteen cages of BALB/cByJ mice (3 to 5 mice per cage) were placed on the Teklad Diet TD 85420 control zinc diet (Table 5.1, Teklad, Madison, WI) for 2 d prior to infection with *S. hyodysenteriae*. Water was supplemented with zinc sulfate heptahydrate to provide the following calculated levels of zinc 12.5, 25, 50, or 100 ppm. Three cages of mice were supplemented with each level of supplemental zinc, four cages of mice received unsupplemented water. Mice were allowed ad libitum access to feed and water.

One cage of mice served as unchallenged controls; the other fifteen cages of mice were challenged with *S. hyodysenteriae* according to the following protocol. The diet was removed 4 h prior to infection with 10^4 spirochetes per mouse. *Serpulina hyodysenteriae* strain B204 was grown as previously described (Nibbelink and Wannemuehler, 1991). Briefly, *S. hyodysenteriae* were grown anaerobically at 37°C in Trypticase Soy Broth (BBL Microbiological Systems, Cockeysville, MD) supplemented with 5% horse serum (Hyclone Laboratoies, Logan, UT), .5% yeast extract (BBL Microbiological Systems), and 1% VPI salt solutions (Solution A: $CaCl_2$, .4 g/L, and $MgSO_4$, .4 g/L; Solution B: $KHPO_4$, 2 g/L; KH_2PO_4, 2 g/L; $NaHCO_3$, 20 g/L; NaCl, 2 g/L). Log phase cultures were obtained. Prior to inoculation into mice, the bacteria were enumerated using a Petroff-Hauser counting chamber, and the bacteria were diluted to the appropriate concentration of organisms with warm complete media. The mice were challenged once with a total

volume of .5 mL culture broth containing 2 x 10^4 organisms per mL. The mice were returned to experimental diet four hours after inoculation and maintained on this diet until the time of necropsy.

Mice were euthanized by cervical dislocation at 3 or 10 d following infection. The ceca were removed, and examined for gross clinical lesions. The cecal tip was removed for histologic evaluation. The ceca were weighed, and cultured for *S. hyodysenteriae*.

Scores were assigned to the cecal lesions as follows: no gross lesions (NGL) = 0; excess cecal mucus with no evidence of cecal atrophy = 1; cecal atrophy and excess mucus localized in the cecal apex only = 2; cecal organ atrophy and excess mucus = 3. Cecal atrophy was determined by comparison of the ceca from infected mice with ceca from uninfected mice.

The histologic evaluation of the ceca examined four parameters: a) the ratio of the gland height to width, b) the number of mitotic figures per 10 glands, c) the amount of epithelial damage and d) the type and relative level of leukocyte infiltration into the lamina propria. Epithelial damage was recorded as none = 0, mild = 1, moderate = 2, or marked = 3. Infiltration of the lamina propria was scored from 0 to 3 as described for the epithelial damage, and an additional point was added for neutrophil infiltration compared with lymphoplasmacytic infiltration.

To determine the number of colony forming units (CFU) per gram of ceca, the ceca were removed aseptically and placed in sterile WhirlPak bags (Baxter Co., Minneapolis, MN). The bags were weighed empty and reweighed after the ceca were placed in the bags. Cecal weight was determined by difference. After weighing, the ceca were suspended in 1:100 (w/v) phosphate-buffered saline (NaCl 136.9 mM, Na_2PO_4 8.1mM, KH_2PO_4 1.5mM, KCl 2.7 mM, pH 7.2) and homogenized in a Stomacher Laboratory Blender (A. J. Stewart Co., London, U.K.). A sample of the suspension was removed and examined by

dark field microscopy for the presence of large spirochetes. Serial dilutions (10-fold) were made of the suspension, and the appropriate dilutions were added to 5 mL molten (45°C) Trypticase Soy agar tubes supplemented with 5% ovine blood and antibiotics (Kunkle and Kinyon, 1988). The tubes were vortexed and poured into 60 mm Petri dishes (#25010 Corning Glass Works, Corning, NY). The plates were incubated anaerobically at 37°C for 96 h. The zones of ß-hemolysin were enumerated and the CFU per g of cecal tissue was calculated.

Statistical analysis of cecal scores for gross clinical lesions and histologic evaluation were made by analysis of variance using the General Linear Model of SAS (1989). The model included level of zinc as the main effect. The experimental unit was a cage of mice.

RESULTS

Ceca from mice euthanized on the third day following infection exhibited no gross clinical signs of disease. *Serpulina hyodysenteriae* was isolated from each infected mouse. There was no significant difference in the number of organisms isolated per gram of ceca at this time point (Table 5.2). The mice receiving 100 ppm of zinc tended to have the lowest average, but the number of organisms isolated from the other groups was extremely variable, ranging from 3,900 to 186,000 organisms per g of cecum.

A summary of the cecal scores of the mice euthanized on day 10 is illustrated in Figure 1. The mice receiving 12.5 ppm supplemental zinc had an average cecal score of .83 compared to 2.83 for the mice on either the no supplemental zinc or the 50 ppm zinc ($P < .05$). Histologic examination of sections of the ceca also demonstrated some differences among treatments. Three days following infection, the zinc supplemented groups had a greater gland height to width ratio compared to the unsupplemented group (Table 5.3). There was no significant difference in the number of mitotic cells per 10 glands, although the unsupplemented mice tended to have fewer mitotic cells. Three days post challenge, only two mice had some mild epithelial damage (1 mouse in the 50 ppm group, and 1 mouse in the 25 ppm group). A mild lymphoplasmacytic infiltration in the lamina propria, was observed in three mice at this time point (1 in each of the 100, 50, and 25 ppm treatment groups). Ten days following challenge the histologic examinations of the ceca show changes associated with development of disease (Table 5.4). These changes were affected by the level of supplemental zinc the mice recieved. A difference in the number of mitotic figures was observed between mice receiving 50 ppm Zn and those receiving 12.5 ppm (Table 5.4, $P < .05$). There was less cellular infiltration into the lamina propria for mice receiving either no supplemental zinc or 12.5 ppm zinc compared with mice

supplemented with 25 ppm or more Zn ($P < .05$). The infiltrating cells were characterized as lymphoplasmacytic for mice supplemented with 12.5 ppm and no added Zn, while mice supplemented with 25 ppm or more Zn had a mixed response in which PMN's were also present. A technical error was made at the 10 d post challenge time point which resulted in incomplete microbiological evaluation of the cecal contents; therefore, the number of organisms per gram ceca could not be compared.

DISCUSSION

Previously, pretreatment with zinc was found to be beneficial against infection with *Francisella tularensis* or *Streptococcus pneumoniae*, but reduced survival in rats exposed to *S. typhimurium* or *Escherichia coli* (Sobocinski et al., 1977a). Administration of zinc in all of these studies was parenteral rather than oral. The data from this experiment indicate that the clinical signs of disease, following infection with *S. hyodysenteriae*, were affected by the level of zinc supplied in the water.

The effect of zinc was dose specific with 12.5 ppm providing the most effective reduction in clinical signs of disease 10 d following challenge. This effect of supplemental zinc seems to have a very narrow dose range. The gross clinical signs of disease were equally severe at 0 and 50 ppm of supplemental zinc. Mice supplemented with 25 ppm of zinc had lesions of intermediate severity between the least severe at 12.5 and the most severe at 0 or 50 ppm of zinc. These results are similar to those described by Snyder and Walker (1976), in which an intraperitoneal dose of .4 mg zinc chloride per mouse effectively protected against a lethal dose of *Salmonella typhosa* endotoxin. This protective effect was substantially reduced when the dose of zinc chloride was varied by as little as .2 mg per mouse.

Since *S. hyodysenteriae* is not a systemic infection, the pathogenesis of the disease is attributed to the development of enteric lesions (Kinyon et al., 1980). The lipopolysaccharide (LPS) of *S. hyodysenteriae* has endotoxin activity (Baum and Joens, 1979; Nuessen et al., 1983) and is important to the development of lesions in mice (Nibbelink and Wannemuehler, 1991). Sobocinski et al.(1977b) demonstrated that intraperitoneal administration of zinc reduced the absorption of ^{51}Cr-labeled endotoxin from the peritoneal cavity of treated mice compared to control mice. The reduced

absorption of endotoxin prevented the increases in the plasma levels of ornithine carbamoyltransferase activity and hepatic tissue necrosis associated with endotoxin administration (Sobocinski et al., 1977b). The effect of supplemental zinc observed in the current study may be due to a similar effect on absorption of endotoxin from the lumen of the intestinal tract.

Histologic examination of ceca sections confirmed that more severe signs of disease occurred in the groups receiving 25, 50, or 100 ppm zinc supplementation compared with groups receiving 12.5 ppm zinc. A difference between the unsupplemented and 12.5 ppm treatment groups was not found in the histologic examination. Paradoxically, it is unclear why less severe signs of inflammation observed in the unsupplemented group resulted in the severe gross clinical signs of disease. Previous reports have indicated a reduction in PMN or macrophage mobilization and phagocytosis when plasma levels of zinc are substantially increased (Chvapil, 1979). The doses of zinc used in this experiment do not seem to have a deleterious effect on cell mobilization. On the contrary, the changes in the inflammatory cells observed in the lamina propria would seem to indicate that zinc supplementation may enhance the infiltration of cells into the lamina propria. Cecal atrophy and mucus production do not appear to be related to the type of inflammatory response in the mice. Histologic changes, observed in the lamina propria of the unsupplemented, 12.5, and 50 ppm mice, do not correlate with the severity of gross cecal lesions that developed. Similar histologic changes were observed in the unsupplemented and 12.5 ppm group, but the unsupplemented group developed severe lesions while the 12.5 ppm group did not develop severe lesions. Furthermore, the mice from the unsupplemented and 50 ppm groups had different histologic changes but both developed severe lesions.

IMPLICATIONS

Zinc may be a factor in the resistance of mice to *S. hyodysenteriae* infection. The effect of zinc supplementation in the drinking water is dose dependent and has a narrow range of effectiveness. It is unknown whether the effect of zinc is on the bacteria itself, the ß-hemolysin, LPS, or on the epithelial cell membrane. However, it appears that adequate zinc nutrition may be beneficial in the prevention or amelioration of enteric disease. The determination of the mechanism involved in this effect will need to be the subject of future studies.

LITERATURE CITED

Albassam, M. A., H. J. Olander, H. L. Thacker, and J. J. Tureck. 1985. Ultrastructural characterization of colonic lesions in pigs inoculated with *Treponema hyodysenteriae*. Can. J. Comp. Med. 49: 384.

Baum, D. H., and L. A. Joens. 1979. Serotypes of beta-hemolytic *Treponema hyodysenteriae*. Infect. Immun. 25: 792.

Chavpil, M., L. Stankova, Z. Bartos, T. Cox., and W. Nichols. 1979. Mobility of peritoneal inflammatory cells after in vivo supplementation with zinc. J. Reticuloendothel. Soc. 25: 345.

Glock, R. D., and D. L. Harris. 1972. Swine dysentery II: Characterization of lesions in pigs inoculated with *Treponema hyodysenteriae* in pure and mixed culture. Vet. Med. Small Anim. Clin. 67: 65.

Hughes, R., H. J. Olander, and C. B. Williams. 1975. Swine dysentery: pathogenicity of *Treponema hyodysenteriae*. Am. J. Vet. Res. 36: 971.

Hyatt, D. R., S. Muir, and L. A. Joens. 1992. Biophysical characterization of the *Serpulina hyodysenteriae* native hemolysin. 73rd Annual Meeting of Conference of Research Workers in Animal Diseases, Chicago, IL. (Abstr.)

Kinyon, J. M., D. L. Harris, and R. D. Glock. 1980. Isolation of *Treponema hyodysenteriae* from experimentally infected pigs at various intervals post inoculation. Proc. Congr. Int. Pig Vet. Soc., Copenhagen.

Kunkle, R. A., and J. M. Kinyon. 1988. Improved selective medium for the isolation of *Treponema hyodysenteriae*. J. Clin. Microbiol. 26: 2357.

Lemcke, R. M., and M. R. Burrows. 1982. Studies on a haemolysin produced by *Treponema hyodysenteriae*. J. Med. Microbiol. 15: 205.

Nibbelink , S. K., and M. J. Wannemuehler. 1991. Susceptibility of inbred mouse strains to infection with *Serpula (Treponema) hyodysenteriae*. Infect. Immun. 59: 3111.

Nibbelink, S. K., and M. J. Wannemuehler. 1992. An enhanced murine model for studies on the pathogenesis of *Serpulina hyodysenteriae*. Infect. Immun. 60: 3433.

Nuessen, M. E., L. A. Joens, and R. D. Glock. 1983. Involvement of lipopolysaccharide in the pathogenicity of *Treponema hyodysenteriae*. J. Immunol. 131: 997.

Saheb, S. A., and L. Lafleur. 1980. Characteristics of the interaction of a treponemal hemolysin with rabbit erythrocytes. Biochimie 62: 787.

Saheb, S. A., L. Massiocotte, and B. Picard. 1980. Purification and characterization of *Treponema hyodysenteriae* hemolysin. Biochimie 62: 779.

SAS. 1989. SAS/STAT® Users Guide: Statistics (Release 6.06) SAS Institute. Inc., Cary, NC.

Sobocinski, P. Z., W. J. Canterbury, and M. C. Powanda. 1977a. Differential effect of parenteral zinc on the course of various bacterial infections. Proc. Soc. Exp. Biol. Med. 156: 334.

Sobocinski, P. Z., M. C. Powanda, W. J. Canterbury, S. V. Machotaka, R. I. Walker, and S. L. Snyder. 1977b. Role of zinc in the abatement of hepatocellular damage and mortality incidence in endotoxemic rats. Infect. Immun. 15: 950.

Snyder, S. L., and R. I. Walker. 1976. Inhibition of lethality in endotoxin-challenged mice treated with zinc chloride. Infect. Immun. 13: 998.

Taylor, D. J., and T. J. L. Alexander. 1971. The production of dysentery in swine by feeding cultures containing a spirochete. Br. Vet. J. 127: viii.

Table 5.1. Composition of the Teklad Diet TD 85420 in grams per kilogram

Ingredient	Control Zinc (g/kg)
Egg white solids, spray dried	200.0
Dextrose, monohydrate	634.2658
Corn oil	100.0
Cellulose (fiber)	29.9107
Zinc carbonate, $ZnCO_3$[b]	.0893
Calcium phosphate, dibasic, $CaHPO_4$	19.767
Magnesium sulfate, $MgSO_4$	2.4752
Potassium chloride, KCl	2.2882
Sodium chloride, NaCl	.7781
Ferrous sulfate, $FeSO_4$•7H2O	.2
Manganese sulfate, MnSO4•H_2O	.1662
Cupric sulfate, $CuSO_4$	.0151
Potassium iodate, KIO_3	.0004
Chromium potassium sulfate, $CrK(SO_4)_2$•$12H_2O$	.02
Vitamin mix, Teklad (#40060)[a]	10.0
Biotin	.004
Ethoxyquin (antioxidant)	.02

[a] Vitamin mix supplies the following grams per kilogram of diet: p-aminobenzoic acid, .11; ascorbic acid, .99; d-biotin, .0004; vitamin B_{12}, .00003; d-calcium pantothenate, .066; choline, 1.43; folic acid, .002; inositol,.11; menadione, .05; niacin, .099; pyridoxine•HCl, .022; riboflavin, .022; thiamin•HCl, .022. The following are supplied in units/kg: vitamin A, 19,824; vitamin D_3, 2,202.5; vitamin E, 121.15.

[b]Provides 46 ppm zinc from zinc carbonate.

Table 5.2. Effect of zinc supplementation on cecal weight and colonization of BALB/cByJ mice infected with *S. hyodysenteriae* three days following infection[a]

Cage Number	Level Zinc in Water, ppm	Day 3 Cecal weight (g)	Day 3 Organisms x 10^4/g ceca
1[b]	None	.13	.0
2	None	.17	131.0
3	None	.15	76.7
4	None	.31	8.7
5	100	.20	91.0
6	100	.17	29.4
7	100	.27	23.0
8	50	.21	171.0
9	50	.19	120.0
10	50	.18	3.9
11	25	.21	16.7
12	25	.17	3.9
13	25	.11	186.0
14	12.5	.12	165.0
15	12.5	.15	24.7
16	12.5	.20	81.3

[a]Number of observations, n = 2 for cage 1, n = 1 for cages 2 to 16. Values for cage 1 are expressed as mean of 2 mice examined.

[b]Unchallenged control cage of mice.

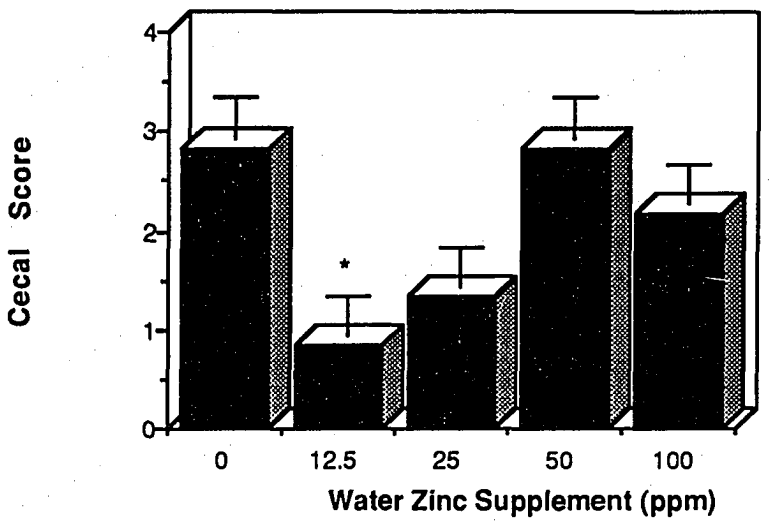

Figure 5.1. Effect of zinc supplementation on development of gross clinical lesions in BALB/cByJ mice infected with *S. hyodysenteriae*. Mice were necropsied 10 d following infection as described in Materials and Methods. Cecal scores range from 0 (no grossly evident lesions) to 3 (severe cecal lesions). Bars indicate pooled standard error. Cecal contents of all challenged mice were darkfield or culture positive for *S. hyodysenteriae*. * $P < .05$.

Table 5.3. Effect of zinc supplementation on cecal histologic parameters of BALB/cByJ mice three days following infection with *S. hyodysenteriae* [a]

Zinc level	Gland H x W	Mitotic figures	Epithelial damage	Lamina propria	Total Score	Summary
None	2	4	0	0	6	normal
None	3	4	0	0	7	normal
None	3	3	0	0	6	normal
MEAN	2.7	3.7	0	0[c]	6.3	
100	4	5	0	1	10	mild inflammation LP cell infiltration
100	4	6	0	0	10	slightly deeper glands
100	3	13	0	0	16	slightly deeper glands
MEAN	3.7[b]	8	0	0[c]	12.3[d]	
50	4	6	0	0	10	slightly deeper glands
50	4	4	0	0	8	slightly deeper glands
50	5	7	1	0	13	slightly deeper glands, mild epithelial changes
MEAN	4.3[b]	5.7	.33	0[c]	10.3	

[a] Abbreviations used: LP = lymphoplasmacytic, mixed indicates lymphoplasmacytic and neutrophilic infiltrating cells, Gland H X W represents the ratio of the gland height to the gland width; scores for epithelial damage and lamina propria inflammation were 0 (no damage), 1 (mild), 2 (moderate), 3 (marked), an additional 2 points was added for mixed cell infiltration. Zinc level = zinc supplemented in water expressed as ppm.

[b] Differ from no supplemental zinc, $P < .05$.

[c] Differ from 25 ppm, $P < .05$.

[d] Differ from no supplemental zinc, $P < .05$.

Table 5.3. Continued

25			•	•	•	insufficient tissue
25	4	7	1	1	13	mild inflammation LP cell infiltration
25	4	4	0	1	9	mild inflammation LP cell infiltration
MEAN	4[b]	5.5	.5	1	11[d]	
12.5	4	8	0	0	12	slightly deeper glands
12.5	4	9	0	0	13	slightly deeper glands
12.5	4	6	0	0	10	slightly deeper glands
MEAN	4[b]	7.7	0	0[c]	11.7[d]	
SE	.32	1.0	.14	.37	1.2	

Table 5.4. Effect of level of supplemental zinc in water on cecal histologic parameters of BALB/cByJ mice ten days following infection with *S. hyodysenteriae*[a]

Zinc level	Gland H x W	Mitotic figures	Epithelial damage	Lamina propria	Total Score	Summary
None	5	8	0	0	13	deeper glands
None	5	5	1	2	13	deeper glands; mild epithelial damage; moderate inflammation; LP cell infiltration
None	5	5	1	1	12	deeper glands; mild epithelial damage; mild inflammation, LP cell infiltration
Mean	5	6^{bc}	.7	1.7^{bcd}	13.3^{bcd}	
100	5	8	1	3	17	deeper glands; mild epithelial damage; mild inflammation, mixed cell infiltration
100	4	10	0	4	18	deeper glands; moderate inflammation, mixed cell infiltration
100	6	14	2	5	27	very deep glands; moderate epithelial damage; marked inflammation, mixed cell infiltration
Mean	5	10.7^{b}	1	4^{be}	20.7^{be}	
50	5	13	2	4	24	deep glands; moderate epithelial damage; moderate inflammation, mixed cell infiltration
50	5	8	3	4	20	deep glands; marked epithelial damage; moderate inflammation, mixed cell infiltration
50	6	12	2	4	24	very deep glands; moderate epithelial damage; moderate inflammation, mixed cell infiltration
Mean	5.3	11^{cd}	2.3	3.7^{d}	22.3^{cf}	

[a] Abbreviations used: LP = lymphoplasmacytic, mixed indicates lymphoplasmacytic and neutrophilic infiltrating cells, Gland H X W represents the ratio of the gland height to the gland width; scores for epithelial damage and lamina propria inflammation were 0 (no damage), 1 (mild), 2 (moderate), 3 (marked), an additional 2 points was added for mixed cell infiltration. Zinc level = zinc supplemented in water expressed as ppm.

[bcdefg]Values within a column with same superscripts differ, ($P < .05$).

Table 5.4. Continued

25	6	12	3	4	25	very deep glands; marked epithelial damage; moderate inflammation, mixed cell infiltration
25	5	10	2	4	21	deep glands; moderate epithelial damage; moderate inflammation, mixed cell infiltration
25	4	8	0	4	12	deeper glands; moderate inflammation, mixed cell infiltration
Mean	5	10	1.7	4[df]	20.7[dg]	
12.5	5	4	1	2	12	deep glands; mild epithelial damage; moderate inflammation LP cell infiltration
12.5	5	9	1	1	16	deep glands; mild epithelial damage; mild inflammation, LP cell infiltration
12.5	6	6	0	1	12	mild deepening of gland; mild inflammation, LP cell infiltration
Mean	5	6.3[d]	.7	2.3[ef]	14[efg]	
SE	.2	1.3	.4	.5	2.1	

GENERAL DISCUSSION

The data presented in this dissertation emphasizes the importance of zinc for adequate immune function in young pigs and identifies zinc as a potentially important factor in the susceptibility to infection with *Serpulina hyodysenteriae*. The effects of zinc deficiency on immune functions in young pigs were characterized using both severe and moderate zinc deficiency models. The importance of zinc in the susceptibility to *Serpulina hyodysenteriae* was studied using both conventional pigs and mice.

The severe zinc deficiency model used produced characteristic signs of deficiency in young pigs including: the presence of parakeratosis and depressed growth, feed intake, and serum zinc. Serum levels of the zinc dependent enzymes, alkaline phosphatase and alanine amino transferase, were also reduced. When compared with zinc supplemented pigs, these animals had lower proliferative response to PWM, but not to PHA. Antibody response to injection with *S. hyodysenteriae* whole cell lysate was lower. The reduction in antibody response was more pronounced for the IgG fraction than for the total immunoglobulin produced. These findings suggest that zinc may be important to helper T cell function in the pig. Specific subsets of the T helper population which promote antibody production and immunoglobulin class switching may be affected more than other T cell populations.

The moderate zinc deficiency model used resulted in a decreased feed intake and growth rate in the pigs but did not produce parakeratosis and did not significantly reduce the levels of alkaline phosphatase. Effects of zinc deficiency were observed on the proliferative response to PWM and ConA. A difference between treatment groups in the proliferative response to PHA was observed before the initiation of dietary treatment. Therefore, it was not possible to evalute the effect of zinc on the response to PHA. The effects of zinc on the proliferatve responses of these pigs were transient, occurring after two weeks of treatment (about 5 to 6 weeks of age). After four weeks of treatment the difference in proliferative

response between deficient and supplemented pigs was not found, but there was a significant difference between the deficient and the supplemented pigs in the percentage of lymphocytes expressing class II, CD2, or CD8 cell surface antigens. Pigs with the moderate zinc deficiency had a lower percentage of MHC class II expressing cells and an increase in the percentage of CD2 and CD8 expressing cells. This may reflect either a specific need for zinc during certain maturational phases of the immune response of young pigs, or may reflect a decreased requirement for zinc with age of the pig.

The importance of zinc on the severity of clinical signs of swine dysentery was evaluated using both pigs and mice. In mice, zinc supplementation of the drinking water resulted in a reduction of clinical signs of disease. The response to zinc was dose specific and the range of effective levels was narrow. This type of response is characteristic of the responses reported by other laboratories examining the effects of zinc on endotoxin challenge. Previous work in our laboratory has examined the importance of LPS as a virulence factor of *S. hyodysenteriae*, and has suggested that an inappropriate inflammatory response to LPS may be an important factor in the initiation of clinical disease. The data, presented in this dissertation, suggests that zinc may be important in protection against the development of clinical disease. The mechanism of this protection is unknown. The narrow range of effectiveness of the supplemental zinc may be a reflection of a multi-factorial pathogenesis for the initiation of this disease. The multi-factorial nature is further emphasized by the variation in the type of cells infiltrating the lamina propria of mice receiving various levels of zinc. Similar gross clinical lesions were observed in mice receiving no supplemental zinc or 50 ppm supplemental zinc. Histologic observations of ceca from these mice indicate that an inflammatory response which results in infiltration of PMNs may not be a predisposing factor necessary to the development of severe clinical signs of disease.

Two experiments were conducted to determine the effects of zinc supplements on the clinical signs of swine dysentery in young pigs. In the first experiment, there was a trend for pigs fed zinc methionine to have fewer clinical signs of disease three and four days following infection compared with pigs fed zinc oxide. In the second experiment, there was no difference between the two zinc supplements but there was a trend for pigs fed either supplement to have fewer signs of disease on the fourth and fifth day after challenge compared with pigs fed an unsupplemented corn and soybean meal diet. The pigs fed zinc methionine had an increased feed intake, and there was a trend for the pigs surviving to have better weight gains and larger thymuses than unsupplemented or zinc oxide supplemented pigs. The differences between these experiments may be due to the levels of zinc supplemented, or the total zinc concentrations in the diets. In the first experiment, pigs fed 40 ppm of supplemental zinc methionine had the fewest signs of disease during the first five days after challenge. The level of supplemental zinc used in the second experiment was 100 ppm. Since the effect of zinc in the drinking water of mice had a narrow range, it may be that this is also true of any beneficial effect of supplemental zinc on clinical signs of disease in pigs.

In addition to the effects on immune function and clinical signs of disease, differences between zinc oxide and zinc methionine were observed in the levels of serum zinc and concentration of zinc in bone ash. In the first experiment, pigs fed zinc methionine had greater concentration of zinc in plasma after 2 wk of dietary treatment compared with pigs fed zinc oxide. Pigs fed 40 ppm of zinc methionine had plasma zinc levels comparable to those of pigs fed 100 ppm zinc oxide. In the third experiment, pigs fed zinc methionine had a greater concentration of serum zinc and zinc in bone ash than pigs fed zinc oxide. Under the conditions of these experiments, the increased levels of zinc in tissues did not correspond to increases in pig performance, either in growth or immune function. These

data do, however, suggest that zinc methionine may be more biologically available than zinc oxide.

The models developed and used in this disertation may be of use in future studies of the effects of zinc on immune functions and disease resistence in mice and swine. The data presented suggests that zinc is important to immune function in swine and may have an effect on clinical signs of swine dysentery. Further, the data suggests that there may be some advantages to using zinc methionine as a supplement compared with zinc oxide in swine feeds.

LITERATURE CITED

Abraham, A., L. N. Mohapatra, K. C. Kandhari, R. K. Pandhi, and L. K. Bhutani. 1975. The effects of some hair oils and unsaturated fatty acids on experimentally induced dermatophytosis. Dermatologica 151: 144.

Albassam, M. A., H. J. Olander, H. L. Thacker, and J. J. Tureck. 1985. Ultrastructural characterization of colonic lesions in pigs inoculated with *Treponema hyodysenteriae*. Can. J. Comp. Med. 49: 384.

Amiraian, K., J. A. McKinney, and L. Duchna. 1974. Effect of zinc and cadmium on guinea pig complement. Immunol. 26: 1135.

Anderson, J. C. 1972. The microanatomy of the lymphnode of germ-free piglets. Br. J. Exp. Pathol. 53: 37.

Auld, D. S., and I. Atsuya. 1976. Yeast RNA polymerase I: a eukaryotic zinc metalloenzyme. Biochem. Biophys. Res. Commun. 69; 548.

Avigan, L. S., and A. W. Bernheimer. 1976. Inhibition by zinc hemolysis induced by bacterial and other cytolytic agents. Infect. Immun. 13: 1378.

Bach, J. F., M. Dardenne, J. M. Pleau, and M. Bach. 1975. Isolation, biochemical characteristics and biological activity of a circulation thymic hormone in the mouse and in the human. Ann. N. Y. Acad. Sci.249: 186.

Barta, O., and N. L. Hubbert. 1978. Testing of hemolytic complement components in domestic animals. Am. J. Vet. Res. 39: 1303.

Bashford, C. L., G. M. Alder, G. Menestrina, K. J. Micklem, J. J. Murphy, and C. A. Pasternak. 1986. Membrane damage by haemolytic viruses, toxins, complement, and other cytotoxic agents: a common mechanism blocked by divalent cations. J. Biol. Chem. 261: 9300.

Baum, D. H., and L. A. Joens. 1979. Serotypes of beta-hemolytic *Treponema hyodysenteriae*. Infect. Immun. 25: 792.

Beach, R. H., M. E. Gershwin, and L. S. Hurley. 1980a. Growth and development of post natally zinc deprived mice. J. Nutr. 110: 201.

Beach R. H., M. E. Gershwin, and L. S. Hurley. 1980b. Impaired immunologic ontogeny in post natal zinc deprivation. J. Nutr. 110: 805.

Beach, R. S., M. E. Gershwin, and L. S. Hurley. 1982. Gestational zinc deprivation in mice: persistence of immunodeficiency for three generations. Science 218: 469.

Bertrand G., and R. C. Bhattacherjee. 1934. L'action combinée du zinc et des vitamins dans l'alimentation des animaux. C. R. Acad. Sci. (Paris) 198: 1823.

Bettger, W. J., and C. B. Taylor. 1986. Effects of copper and zinc status on the concentration of copper and zinc in the erythrocyte membrane. Nutr. Res. 6: 451.

Binns, R. M., R. Pabst, and S. T. Licence. 1986. The behavior of pig lymphocyte populations in vivo. Swine Biomed. Res. 3: 1837.

Boyle, M. D., J. J. Langone, and T. Borsos. 1979. Studies on the terminal stages of immune hemolysis. IV. Effects of metal salts. J. Immunol. 122: 1209.

Chandra, R. K. 1984. Excessive intake of zinc impairs immune responses. J. Am. Med. Assoc. 252: 1443.

Chesters, J. K., and M. Will. 1981a. Zinc transport proteins in plasma. Br. J. Nutr. 46: 111.

Chesters, J. K., and M. Will. 1981b. Measurement of zinc flux through plasma in normal and endotoxin-stressed pigs and the effects fo Zn supplementation during stress. Br. J. Nutr. 46: 119.

Chia, S. P., and D. J. Taylor. 1978. Factors affecting the survival of *Treponema hyodysenteriae* in dysenteric pig feces. Vet. Rec. 103: 68.

Chirase, N. K., D. P. Hutcheson, G. B. Thompson. 1991. Feed intake, rectal temperature, and serum mineral concentrations of feedlot cattle fed zinc oxide or zinc methionine and challenged with infectious bovine rhimotracheitis virus. J. Anim. Sci. 69: 4137.

Clejan, S., V. T. Maddaiah, M. Castro-Magana, and P. J. Collipp. 1981. Zinc deficiency-induced changes in the composition of microsomal membranes and the enzymatic regulation of glycerolipid synthesis. Lipids 16: 454.

Coles, E. H. 1974. In: Veterinary Clinical Pathology. p 40. W. B. Saunders Company. Philadelphia, PA.

Cousins, R. J. 1978. Synthesis and degredation of hepatic zinc-thionein and its role in zinc metabolism, In: M. Kirchgessner (Ed.) Proc. 3rd Intl. Symp. on Trace Element Metabolism in Man and Animals , p. 57 Arbeitskreis fur Tierenahrungsforschung Weihenstephan, West Germany.

Cousins, R. J. 1985. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplamin. Physiol. Rev. 65: 238.

Cousins, R. J., and A. S. Leinhart. 1988. Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin-1. FASEB J. 2: 2884.

Crossley, L. G. and R. R. Proter. 1980. Purification of the human complement control protein C3b inactivator. Biochem. J. 191: 173.

Csermely, P., and J. Somogyi. 1989. Zinc as a possible mediator of signal transduction in T lymphocytes. Acta Physiol. Hung. 75: 195.

Cunningham, B. C., S. Bass, and G. Fuh. 1990. Zinc mediation of the binding of human growth hormone to the human prolactin receptor. Science 250: 1709.

Curtis, J., and F. J. Bourne. 1971. Immunoglobulin quantitation in sow serm, colostrum, and milk and the serum of young pigs. Biochim. Biophys. Acta. 236: 319.

Dahmer, E. J., B. W. Coleman, R. H. Grummer, and W. G. Hoekstra. 1972. Alleviation of parakeratosis in zinc-deficient swine by high levels of dietary histidine. J. Anim. Sci. 35: 1181.

Davies, N. T. 1980. Studies in the absorption of zinc by rat intestine. Br. J. Nutr. 43: 189.

Dib, A., and J-P. Carreau. 1985. Effects of gamma-linolenic acid supplementation on lipogenesis regulation in the pregnant zinc deficient rat and fetus. Int. J. Biochem. 18: 1503.

Dinarello, C. A. 1988. Biology of interleukin 1. FASEB J. 2: 108.

DiSilvestro, R. A., and R. J. Cousins. 1984. Mediation of endotoxin-induced changes in zinc metabolism in rats. Am. J. Physiol. 247: E436.

Dowd, P. S., J. Kelleher, and P. J. Guillou. 1986. T-lymphocyte subsets and interleukin-2 production in zinc-deficient rats. Br. J. Nutr. 55: 59.

Dreosti, I. E., S. H. Tas, and L. S. Hurley. 1968. Plasma zinc and leukocyte changes in weanling and pregnant rats during zinc deficiency. Proc. Soc. Exp. Med. 128: 169.

Duncan, I. A., R. M. Binns, and W. P. H. Duffus. 1989. The null T cell in pig blood is not an NK cell. Immunol. 68: 392.

Egan, L. T. 1981. The epidemiological aspests of swine dysentery in the midwestern United States. Ph.D. Dissertation., Iowa State University, Ames, Iowa.

Evans, B. D., and K. Amiraian. 1984. Interaction of Zn^{2+} with guinea-pig C5 convertase and guinea-pig C5. Molec. Immun. 21: 69.

Evans, B. D., and K. Amiraian. 1987. Effect of zinc on lysis of sheep erythrocytes by guinea pig C4-deficient serum. Molec. Immun. 24: 1345.

Evans, G. W., C. I. Grace, and H. J. Votava. 1975. A proposed mechanism for zinc absorption in the rat. Am. J. Physiol. 228: 501.

Evans, G. W., and P. E. Johnson. 1978. Copper - and zinc - binding ligands in the intestinal mucosa, In:, M. Kirchgessner (Ed.), Proc. 3rd Intl. Symp. on Trace element Metabolism in Man and Animals Arbeitskreis fur Tierernahrungs forschung, Weihenstephan, West Germany p. 98.

Fraker, P. J., S. M. Haas and R. W. Luecke. 1977. Effect of zinc deficiency on the immune response of the adult A/J mouse. J. Nutr. 107: 1889.

Franek, F., and I. Riha. 1964. Purification and structural characterization of 5S gamma globulin in new born pigs. Immunochemistry 1: 49.

Franek, F., I. Riha, and J. Sterzl. 1961. Characteristics of gamma globulin, lacking antibody properties in newborn pigs. Nature 189: 1020.

Franek, F., V. Joua, and J. Kostka. 1962. The nature of pig antibodies against *Brucella suis*. Folia Microbiol. (Prague) 7:1.

Frost, P., J. C. Chen, I. Rabbani, J. Smith, and A. S. Prasad. 1977. The effect of zinc deficiency on the immune response. In: G. J. Brewer (Ed.) "Zinc Metabolism: Current Aspects in Health and Disease" Alan R. Liss, New York.

Galdes, A., and B. L. Vallee. 1983. Categories of zinc metalloenzyme. In: H. Siegel (Ed.) "Metal Ions in Biological Systems, Vol. 15: Zinc and It's Role in Biology and Nutrition." Marcel Dekker, Inc., New York

Glock, R. D., and D. L. Harris. 1972. Swine dysentery II: Characterization of lesions in pigs inocculated with *Treponema hyodysenteriae* in pure and mixed culture. Vet. Med. Small Anim. Clin. 67: 65.

Glock, R. D., K. J. Vanderloo, and J. M. Kinyon. 1975. Survival of certain pathogenic organisms in swine lagoon effluent. J. Am. Vet. Med. Assoc. 166: 277.

Glock, R. D., K. J. Schwartz, and D. L. Harris. 1978. Parenteral immunization of pigs against infection with *Treponema hyodysenteriae*. Am. J. Vet. Res. 39: 639.

Götze, O., I. Haupt, and H. Fischer. 1968. Immune haemolysis reaction of the terminal complement component. Nature 217: 1165.

Greer, J. M., and M. J. Wannemuehler. 1989a. Pathogenesis of *Treponema hyodysenteriae*: induction of interluekin-1 and tumor necrosis factor by a treponemal butanol/water extract (endotoxin). Microbiol. Pathogen. 7: 279.

Greer , J. M., and Wannemuehler. 1989b. Comparison of the biological responses induced by lipopolysaccharide and endotoxin of *Treponema hyodysenteriae* and *Treponema innocens*. Infect. Immun. 57: 717.

Haas, S. M., P. J. Fraker, and R. W. Luecke. 1976. The effect of zinc deficiency on the immune response of A/J mice. Fed. Proc. 35: 659.

Hampson, D. J., R. Cutler, and B. J. Lee. 1992. Virulent *Serpulina hyodysenteriae* from a pig in a herd free of clinical swine dysentery. Vet. Rec. 131: 318.

Harris, D. L., T. J. L. Alexander, S. C. Whipp, I. M. Robinson, R. D. G.ock, and P. J. Matthews. 1978. Swine dysentery: studies of gnotobiotic pigs inoculated with *Treponema hyodysenteria, Bacteroides vulgatus,* and *Fusobacterium necrophorum*. J. Am. Vet. Med. Assoc. 172: 468.

Harris, D. L., R. D. Glock, C. R. Christensen, and J. M. Kinyon. 1972. Swine dysentery-I. Inoculation of pigs with *Treponema hyodysenteriae* (new species) and reproduction of the disease. Vet. Med. Small Anim. Clin. 67: 61.

Hempe, J. M., and R. J. Cousins. 1989. Effect of EDTA and zinc methionine complex on zinc absorption by rat intestine. J. Nutr. 119: 1179.

Hempe, J. M., and R. J. Cousins. 1991. Cysteine-rich intestineal protein binds zinc during transmucosal zinc transport. Proc. Natl. Acad. Sci. USA. 88: 9671.

Hempe, J. M., and R. J. Cousins. 1992. Cysteine-rich intestinal protein and intestinal metallothionein: an inverse relationship as a conceptual model for zinc absorption in rats. J. Nutr. 122: 89.

Hill, D. A., E. R. Peo, Jr., A. J. Lewis, and J. D. Crenshaw. 1986. Zinc-amino acid complexes for swine. J. Anim. Sci. 63: 121.

Hill, G. M., and E. R. Miller. 1983. Effect of dietary zinc levels on growth and development of the gilt. J. Anim. Sci. 57: 106.

Hill, G. M., E. R. Miller, and H. D. Stowe. 1983. Effect of dietary zinc levels on health and productivity of gilts and sows through two parities. J. Anim. Sci. 57: 114.

Hoadley, J. E., A. S. Leinart, and R. J. Cousins. 1987. Kinetic analysis of zinc uptake and serosal transfer by a vascularly perfused rat intestine. Am. J. Physiol. 252: G825.

Hoadley, J. E., A. S. Leinart, and R. J. Cousins. 1988. Relationship of ^{65}Zn absorption kinetics to intestinal metallothionein in rats: effects of zinc depletion and fasting. J. Nutr. 118: 497.

Hoekstra, W. G., E. C. Faltin, C. W. Lin, H. F. Roberts, and R. H. Grummer. 1967. Zinc deficiency in reproducing gilts fed a diet high in calcium and its effect on tissue zinc and blood serum alkaline phosphatase. J. Anim. Sci. 26: 1348.

Hoekstra, W. G., P. K. Lewis, P. H. Phillips, and R. H. Grummer. 1956. The relationship of parakeratosis, supplemental calcium, and zinc content of certain body components of swine. J. Anim. Sci. 15: 752.

Hume, D. A., and W. F. Doe. 1988. Role of macrophages in cellular defense. In: M. F. Heyworth and A. I. Jones (Eds.) "Immunology of the Gastrointestinal Tract and Liver." Raven Press Ltd., New York.

Hyatt, D. R., S. Muir, and L. A. Joens. 1992. Biophysical characterization of the *Serpulina hyodysenteriae* native hemolysin. 73rd Annual Meeting of Conference of Research Workers in Animal Diseases, Chicago, IL. (Abstr.)

James, S. J., M. Swendseid, and T. Makinodan. 1987. Macrophage-mediated depression of T-cell proliferation in zinc-deficient mice. J. Nutr. 117: 1982.

Joens, L. A., and R. D. Glock. 1979. Experimental infection in mice with *Treponema hyodysenteriae* Infect. Immun. 25: 757

Joens, L. A., R. D. Glock, S. C. Whipp, I. M. Robinson, and D. L. Harris. 1981. Location of *Treponema hyodysenteriae* and synergistic anaerobic bacteria in colonic lesions of gnotobiotic pigs. Vet. Microbiol. 6: 69.

Joens, L. A., and J. M. Kinyon. 1982. Isolation of *Treponema hyodysenteriae* from wild rodents. J. Clin. Microbiol. 15: 994.

Joens, L. A., J. G. Songer, D. L. Harris and R. D. Glock. 1978. Experimental infection with *Treponema hyodysenteriae* in guinea pigs. Infect. Immun. 22: 132.

Johanning, G. L., and B. L. O'Dell. 1989. Effect of zinc deficiency and food restriction in rats on erythrocyte membrane zinc, phospholipid and protein content. J. Nutr. 119: 1654.

Kalinowski, J., and E. R. Chavez. 1984. Effect of low dietary zinc during late gestation and early lactation on the sow and neonatal piglets. Can. J. Anim. Sci. 64: 749.

Keilin, D., and J. Mann. 1940. Carbonic anhydrase. Purfication and nature of the enzyme. Biochem. J. 34: 1163.

Kennedy, M. J., D. K. Rosnick, R. G. Ulrich, and R. J. Yancey. 1988. Association of *Treponema hyodysenteriae* with porcine intestinal mucosa. J. Gen. Microbiol. 134: 1565.

Kent, K. A., and R. M. Lemcke. 1984. Purification and cytotoxic activity of a haemolysin produced by *Treponema hyodysenteriae*. In: Abstracts of the International Pig Veterinary Society, Ghent, Belgium. p. 785.

Kernkamp, H. C. H., and E. F. Ferrin. 1953. Parakeratosis in swine. J. Am. Vet. Med. Assoc. 123: 217.

Kim, Y. B., S. G. Bradley, and D. W. Watson. 1966. Ontogeny of the immune response. I. Development of immunoglobulins in germfree and conventional colostrum-deprived piglets. J. Immunol. 97: 52.

Kinoshita, T. 1991. Biology of complement: the overture. Immunology Today. Vol. 12, No. 9 : 291.

Kinyon, J. M., D. L. Harris, and R. D. Glock. 1980. Isolation of *Treponema hyodysenteriae* from experimentally infected pigs at various intervals post inoculation. Proc. Int. Congr. Pig Vet. Soc., Copenhagen.

Kirchgessner M., and H. P. Roth. 1980. Biochemical changes of hormones and metalloenzymes in zinc deficiency. In: J. O. Nriagu (Ed.) "Zinc in the enviornment, Part II: Health Effects." Wiley, New York.

Kligman, A. M., and D. Ginsberg. 1950. Immunity of the adult scalp to infection with *Microsporum audouini* J. Invest. Dermatol. 14: 345.

Ku, P. K., D. E. Ullrey, and E. R. Miller. 1970. Zinc deficiency and tissue nucleic acid and protein concentration. In: C.F. Mills (Ed.) "Trace Element Metabolism in Animals". E. and S. Livingstone Edinburugh. and London.

Kuper, C. F., P. J. Koornstra, D. M. H. Hameleers, J. Biewenga, B. J. Spit, A. M. Duijvestijn, P. J. C. van Breda Vriesman, and T. Sminia. 1992. The role of nasopharyngeal lymphoid tissue. Immunol. Today 13: 219.

LaFuse, W. P., and C. S. David. 1984. Ia antigens, genes, molecules, and function. Transplantation 38:443.

Lemcke, R. M., and M. R. Burrows. 1982. Studies on a haemolysin produced by *Treponema hyodysenteriae*. J. Med. Microbiol. 15: 205.

Liptrap, D. O., E. R. Miller, D. E. Ullery, D. L. Whitenack, B. L. Schoepke, and R. W. Luecke. 1970. Sex influence on the zinc requirement of developing swine. J. Anim. Sci. 30: 736.

Luecke, R. W., J. A. Hoeffer, W. S. Brammel, and D. A. Schmidt. 1957. Calcium and zinc in parakeratosis of swine. J. Anim. Sci. 16: 3.

Luecke, R. W., C. E. Simonel, and P. J. Fraker. 1978. The effect of restricted dietary intake on the antibody mediated response of the zinc deficient A/J mouse. J. Nutr. 108: 881.

Lunney, J. K., and M. D. Pescovitz. 1987. Phenotypic and functional characterization of pig lymphocyte populations. Vet. Immunol. Immunopathol. 17: 135.

Lysons, R. J., K. A. Kent, A. P. Bland, R. Sellwood, W. F. Robinson, and A. J. Frost. 1991. A cytotoxic haemolysin from *Treponema hyodysenteriae* - a probable virulence determinant in swine dysentery. J. Med. Microbiol. 34: 97.

Macapinlac, M. P., W. N. Pearson, and W. J. Darby. 1966. Some characteristics of zinc deficiency in the albino rat. In: A. S. Prasad (Ed.) "Zinc Metabolism" Charles Thomas, Springfield, IL.

Menard, M. P., and R. J. Cousins. 1983. Effect of citrate, glutathione and picolinate on zinc transport by brush border membrane vesicles from rat intestine. J. Nutr. 113: 1653.

Metzger, J. J., and M. Fougereau. 1968. Characterizations biochimiques des immunoglobulines gamma-G et gamma-M porcines. Rech. Vet. 1: 37.

Meyer, R. C., J. Simon, and C. S. Byerly. 1974a. The etiology of swine dysentery. I. Oral inoculation of germ-free swine with *Treponema hyodysenteriae* and *Vibrio coli*. Vet. Path. 11: 515.

Meyer, R. C., J. Simon, and C. S. Byerly. 1974b. The etiology of swine dysentery II. Effect of a known microbial flora, weaning and diet on disease production in gnotobiotic and conventional swine. Vet. Path. 11: 527.

Meyer, R. C., J. Simon, and C. S. Byerly. 1975. The etiology of swine dysentery III. The role of selected gram negative abligate anaerobes. Vet. Path. 12: 46.

Miller, J., A. D. McLachlan, and A. Klug. 1985. Repetitive zinc-binding domains in the protein transcription factor IIIA from *Xenopus* oocytes. EMBO J. 4: 1609.

Miller, E. R., D. O. Liptrap and D. E. Ullrey. 1970. Sex influence on zinc requirements of swine. In: C.F. Mills (Eds) "Trace Element Metabolism in Animals". E. and S. Livingstone Edinburugh. and London.

Miller, E. R., R. W. Luecke, D. E. Ullrey, B. V. Battzer, B. L. Bradley, and J. A. Hoeffer. 1968. Biochemical, skeletal, and allometric changes due to zinc deficiency in the baby pig. J. Nutr. 95: 278.

Miller, E. R., H. D. Stowe, P. K. Ku, and G. M. Hill. 1979. Copper and zinc in swine nutrition. In: "National Feed Ingredients Association Literature Review on Copper and Zinc in Animal Nutrition." National Feed Ingredients Association. West Des Moines, IA:

Miller, W. J. 1969. Absorption, tissue distribution, endogenous, excretion and homeostatic control of zinc in ruminants. Amer. J. Clin. Nutrition 22: 1323.

Miller, W. J., R. L. Kincaid, M. W. Neathery, R. P. Gentry, M. S. Ansari, and J. W. Lassiter. 1978. Zinc metabolism in calves, cows, rats and chicks fed high dietary zinc. Proc. 3rd Int. Symp. on Trace Element Metabolism in Man and Animals. July 25-29, 1977, West Germany, 175.

Montgomery, D. W., M. Chvapil, and C. F. Zukoski. 1979. Effect of zinc chloride on guinea pig complement component activity *in vitro* : concentration dependent inhibition and enhancement. Infect. Immun. 23: 424.

Mooradian, A. D., and M. K. Song. 1987. The intestinal zinc transport in aged rats. Mech. Ageing Dev. 41: 189.

Nash, L., T. Iwata, G. Fernandes, R. A. Good, and G. Incefy. 1979. Effect of zinc deficiency on autologous rosette-forming cells. Cell. Immunol. 48: 238.

Nibbelink, S. K. 1992. The role of tumor necrosis factor and secondary bacteria in the pathogenesis of swine dysentery. Ph. D. Dissertation. Iowa State University, Ames, IA.

Nibbelink , S. K., and M. J. Wannemuehler. 1991. Susceptiblity of inbred mouse strains to infection with *Serpula (Treponema) hyodysenteriae*. Infect. Immun. 59:3111

NRC. 1988. Nutrient Requirements of Swine. (9th Ed.). National Academy Press, Washington , D.C.

Nuessen, M. E., L. A. Joens, and R. D. Glock. 1983. Involvement of lipopolysaccharide in the pathogenicity of *Treponema hyodysenteriae*. J. Immunol. 131: 997.

O'Dell, B. L. 1992. Cysteine-rich intestinal protein (CRIP): a new intestinal zinc transport protein. Nutr. Rev. 50 (8): 232.

O'Dell, B. L., J. D. Browning, and P. G. Reeves. 1987. Zinc deficiency increases the osmotic fragility of rat erythrocytes. J. Nutr. 117: 1883.

Osler, A. G., 1976. In: A. G. Osler, and L. Weiss. (Eds) Complement: Mechanisms and functions. Prentice-Hall, Inc., Englewood Cliffs, N. J.

Owen, A. A., E. R. Peo, Jr., P. J. Cunningham, and B. D. Moser. 1973. Effect of EDTA on utilization of dietary zinc by G-F swine. J. Anim. Sci. 37: 470.

Owen, W. J. 1986. National Animal Health Monitoring System. USDA-APHIS and Iowa High Technology Council. College of Veterinary Medicine, Iowa State University, Ames, Iowa.

Pabst, R., and R. M. Binns. 1986. Comparison of lymphocyte production and migration in pig lymphnodes, tonsils, spleen, bone marrow and thymus. Swine Biomed. Res. 3: 1865.

Pan, T. and J. E. Coleman. 1990. GAL4 transcription factor is not a "zinc finger" but forms a $Zn(II)_2$ Cys_6 binuclear cluster. Proc. Natl. Acad. Sci. USA 87: 2007.

Pan, T., L. P. Freeman, and J. E. Coleman. 1990. Cadmium-113 NMR studies of the DNA binding domain of the mammalian glucocorticoid hormone receptor. Biochem. 29: 9218.

Pasternak, C. A., G. M. Alder, C. L. Bashford, C. L. Buckley, K. J. Micklem, and K. Patel. 1985. Cell damage by viruses, toxins, and complement: common features of pore formation and its inhibition by Ca2+. In: C. A. Pasternak and P. J. Quinn (Ed.) "The molecular basis of movement through membranes. Biochem. Soc. Symp. 50: 247.

Pearson, W. N., T. Schwink, M. Reich. 1966. In vitro studies of zinc absorption in the rat. In: A. S. Prasad. (Ed.) Zinc Metabolism. Charles C. Thomas, Springfield, Illinois. p. 239.

Pescovitz, M. D., J. K. Lunney, D. H. Sachs. 1984. Preparation and characterization of monoclonal antibodies reactive with PBL. J. Immunol. 133: 368.

Pescovitz, M. D., J. K. Lunney, D. H. Sachs. 1985. Murine anti-T4 and T8 monoclonal antibodies. Distribution and effects on proliferation and cytotoxic T cells. J. Immunol. 134: 37.

Pond, W. G., and J. R. Jones. 1964. Effect of level of zinc in high calcium diets on pigs from weanling through one reproduction cycle and on the growth of their offspring. J. Anim. Sci. 23: 1057.

Porter, P. 1969. Transfer of immunoglobulins IgG, IgA, and IgM to lacteal secretions in the parturient sow and their absorption by the neonatal piglet. Biochim. Biophys. Acta 181: 381.

Porter, P., and W. D. Allen. 1972. Classes of Immunoglobulins related to immunity in the pig. J. Am. Vet. Med. Assoc. 160: 511.

Prasad, A. S., D. Oberlas, E. R. Miller, and R.W. Luecke. 1971. Biochemical effects of zinc deficiency: changes in activities of zinc dependent enzymes and ribonucleic acid and deoxyribonucleic acid content of tissues. J. Lab. Clin. Med. 77: 144.

Prasad, A. S., D. Oberleas, P. Wolf, J. P. Horwitz, E. R. Miller, and R. W. Luecke. 1969. Changes in trace elements and enzyme activities in tissues of zinc deficient pigs. Am. J. Clin. Nutr. 22: 628.

Quarterman, J. 1974. The effects of zinc deficiency or excess in the rat. In: W. G. Hoekstra (Ed.). "Trace Element Metabolism in Animals-2". University Park Press, Baltimore.

Raulin, J. 1869. Estudes cliniques sur la vegetation. Ann. Sci. Nat. Botan. Biol. Vegetale (5) 11: 93.

Richards, M. P., and R. J. Cousins. 1976. Zinc-binding protein: relationship to short term changes in zinc metabolism. Proc. Soc. Expt. Biol. Med. 153: 52.

Roncalli, R. A., and W. H. D. Leaning. 1976. Geographical distribution of swine dysentery. Proc. Int. Congr. Pig Vet. Soc. Ames, L17.

Rothman, S., A. Smiljanic, A. L. Shapiro, and A. W. Weitkamp. 1947. The spontaneous cure of tinea capitis in pubertiy. J. Invest. Dermatol. 8: 81.

Rowley, D., and K. J. Turner. 1964. Increase in macroglobulin antbodies of mouse and pig following injection of bacterial lipopolysaccharide. Immunology 7: 394.

Saheb, S. A., and L. Lafleur. 1980. Characteristics of the interaction of a treponemal hemolysin with rabbit erythrocytes. Biochimie 62: 787.

Saheb, S. A., L. Massiocotte, and B. Picard. 1980. Purification and characterization of *Treponema hyodysenteriae* hemolysin. Biochimie 62: 779.

Scuderi, P. 1990. Differential effects of copper and zinc on human perpheral blood monocyte cytokine secretion. Cell. Immunol. 126: 391.

Shanklin, S. H., E. R. Miller, D. E. Ullrey, J. A. Hoefer, and R. W. Luecke. 1968. Zinc requirement of baby pigs on casein diets. J. Nutr. 96: 101.

Shonle, P. G., 1988. Dermatophytosis. In: "Immunology of the Fungal Diseases." (Ed.) R. A. Cox. p. 2. CRC Press, Inc., Boca Raton, FL.

Song, M. K. and N. F. Adham. 1977. Prostaglandin regulation of zinc absorption in rats. Fed. Proc. 36: 1138.

Song, M. K. and N. F. Adham. 1986. Evidence for an important role of prostaglandins E_2 and F_2 in the regulation of transport in the rat. J. Nutr. 116: 2152.

Songer, J. G., and D. L. Harris. 1978. Transmission of swine dysentery by carrier pigs. Am. J. Vet. Res. 39: 913.

Songer, J. G., R. D. Glock, K. J. Schwartz, and D. L. Harris. 1978. Isolation of *Treponema hyodysenteriae* from sources other than swine. J. Am. Vet. Med. Assoc. 172: 464.

Stanton, T. B. 1992. Proposal to change the genus designation *Serpula* to *Serpulina* gen. nov. containing the species *Serpulina hyodysenteriae* comb. nov. and *Serpulina innocens* comb. nov. Int. J. Syst. Bacteriol. 42: 189.

Stanton, T. B., N. S. Jensen, T. A. Casey, L. A. Tordoff, F. E. Dewhirst, and B. J. Paster. 1991. Reclassification of *Treponema hyodysenteriae* and *Treponema innocens* in a new genus, *Serpula* gen. nov., as *Serpula hyodysenteriae* comb. nov. and *Serpula innocens* comb. nov. Int. J. Syst. Bact. 41: 50.

Steel, L., and R. J. Cousins. 1985. Kinetics of zinc absorption by luminally and vascularly perfused rat intestine. Am. J. Physiol. 248: G46.

Stertzl, J., J. KOstka, I. Rhia, and I. Mandel. 1960. Attempts to determine the formation and character of globulin and of natural and immune antibodies in young pigs reared without colostrum. Folia Microbiol. 5: 29.

Suenaga, I., and T. Yamazaki. 1983. Experimental infection with *Treponema hyodysenteriae* in nude mice. Zbl. Bakt. Hyg. I Abt. Org. A 254: 528.

Sueyoshi, M., and Y. Adachi. 1990. Diarrhea induced by *Treopnema hyodysenteriae* : a young chick cecal model for swine dysentery. Infect. Immun. 58: 3348.

Taylor, D. J., and T. J. L. Alexander. 1971. The production of dysentery in swine by feeding cultures containing a spirochete. Br. Vet. J. 127: viii.

Taylor, D. J. and W. F. Blakemore. 1971. Spirochaetal invasion of the colonic epithelium in swine dysentery. Res. Vet. Sci. 12: 177.

Teige, J., T. Landsverk, A. Lund, and H. J. Larsen. 1981. Swine dysentery: a scanning electron microscopy investigation. Acta. Vet. Scand. 22: 218.

Theuer, R. C. and W. G. Hoekstra. 1966. Oxidation of ^{14}C-labeled carbohydrate, fat, and amino acid substrates by zinc-deficient rats. J. Nutr. 89: 448.

Todd, W. R., C. A. Elvehjem, and E. B. Hart. 1934. Zinc in the nutrition of the rat. Am. J. Physiol. 107: 146.

Tucker, H. F., and W. D. Salmon. 1955. Parakeratosis or zinc deficiency disease in the pig. Proc. Soc. Exp. Biol. Med. 88: 613.

Underwood, E. J. 1977. Trace Elements in Human and Animal Nutrition, 4th ed., Academic Press, New York.

Vallee, B. L., J. E. Coleman, and D. S. Auld. 1991. Zinc fingers, zinc clusters and zinc twists in DNA-binding protein domains. Proc. Natl. Acad. Sci. USA 88: 999.

Vallee, B. L., and A. Galdes. 1984. The metallobiochemistry of zinc enzymes. Adv. Enzymol. 56: 283.

Vruwink, K. G., M. P. Fletcher, C. L. Keen, M. S. Golub, A. G. Hendrickx, and M. E. Gershwin. 1991. Moderate zinc deficiency in rhesus monkeys. An intrinsic defect of neutrophil chemotaxis corrected by zinc repletion. J. Immunol. 146: 244.

Warner, G. L., and D. A. Lawrence. 1986. Stimulation of murine lymphocyte responses by cations. Cell. Immunol. 101: 425.

Whipp, S. C., J. Pohlenz, D. L. Harris, I. M. Robinson, R. D. Glock, and R. Kunkle. 1982. Pathogenicity of *Treponema hyodysenteriae* in uncontaminated gnotobiotic pigs. In: 7th Int. Pig Vet. Soc. Congress, p. 27. Mexico City, Mexico.

Whipp, S. C., I. M. Robinson, D. L. Harris, R. D. Glock, P. J. Mathews, and T. J. L. Alexander. 1979. Pathogenic synergism between *Treponema hyodysenteriae* and other selected anaerobes in gnotobiotic pigs. Infect. Immun. 26: 1042.

Whitenack, D. C., C. K. Whitehair, and E. R. Miller. 1978. Influence of enteric infection on zinc utilization and clinical signs and lesions of zinc deficiency in young swine. Amer. J. Vet. Res. 39: 1447.

Whiting, R. A., L. P. Doyle, and R. S. Spray. 1921. Swine dysentery. Purdue Univeristy Agric. Exper. Station Bul. #257.

Widdowson, E. M., and J. W. T. Dickerson. 1964. Chemical composition of the body. In: Comar and Bronner (Eds) "Mineral Metabolism, Vol. 2A" Academic Press, New York.

Williams, R. J. P. 1984. Zinc: what is its role in biology? Endeavour 8: 65.

Winchurch, R. A. 1988. Activation of thymocyte responses to interleukin-1 by zinc. Clin. Immunol. Immunopath. 47: 174.

Winchurch, R. A., J. Togo, and W. H. Adler. 1987. Supplemental zinc (Zn^{2+}) restores antibody formation in cultures of aged spleen cells II. Effects on mediator production. Eur. J. Immunol. 17: 127.

Yamamoto, K., and M. Takahashi. 1975. Inhibition of the terminal stage of complement-mediated lysis (reactive lysis) by zinc and copper ions. Int. Arch. Allergy Appl. Immunol. 48: 653.

Zuckerman, F. A., and R. J. Husman. 1992. Porcine CD4+CD8+ double positive lymphocytes represent primed T-cells and have a memory T-cell phenotype, $CD29^{bright}$ $LFA\text{-}1^{bright}$. 73rd Annual Meeting of the Conference of Research Workers in Animal Diseases, Chicago, IL. (Abstr.)

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APPENDIX: ANALYSIS OF VARIANCE TABLES

Table A.1. Analysis of variance of pig performance, and plasma zinc levels for the first week. Paper 1

		Mean Squares			
Source	df	ADG, kg	ADFI, kg	Gain/feed	Plasma Zn, mg/dL
Block	3	.042	.076	.014	.011
Treatment	3	.028	.062	.019	.031
Error	9	.011	.019	.017	.016
Total	15	.020	.039	.017	.018
Contrasts					
Sources	1	.031	.044	.027	.010
Levels	1	.044	.054	.030	.053
Source x level	1	.038	.086	.027	.005

Table A.2. Analysis of variance of pig performance, and plasma zinc levels for the second week. Paper 1

		Mean Squares				
Source	df	ADG, kg	ADFI, kg	Gain/feed	Plasma Zn, mg/dL[a]	GSH Px, units/mL
Block	3	.017	.078	.015	.024	.026
Treatment	3	.005	.010	.019	.034	.019
Error	9	.009	.017	.017	.009	.030
Total	15	.010	.028	.017	.018	.027
Contrasts						
Sources	1	.000	.006	.027	.044	.030
Levels	1	.001	.000	.030	.046	.000
Source x level	1	.001	.015	.027	.035	.055

[a]df = 14.

Table A.3. Analysis of variance of pig performance, plasma zinc levels, and glutathione peroxidase activity for the third week. Paper 1

		Mean Squares			
Source	df	ADG, kg	ADFI kg	Plasma Zn, mg/dL	GSH Px, units/mL
Block	3	.001	.064	.082	.441
Treatment	3	.001	.277[a]	.148	.104
Error	4	.001	.044	.106	.039
Total	10	.001	.113[b]	.165	.179
Contrasts					
Sources	1	.003	.567[c]	.063	.129
Levels	1	.000	.016	.055	.083
Source x level	1	.002	.729[d]	.150	.018

[a] $P < .04$.

[b] df = 11.

[c] $P < .02$.

[d] $P < .01$.

Table A.4. Analysis of variance of clinical signs of swine dysentery. Paper 1

Source	df	Mean Squares					
		Mortality	Clinical Score 1d	Clinical Score 2d	Clinical Score 3d	Clinical Score 4d	Clinical Score 5d
Block	3	.229	.896	3.896	8.167[a]	9.167[b]	2.5625
Treatment	3	.563	.896	1.729	3.333	2.000	1.229
Error	9	.174	1.118	4.507	1.944	1.055	1.063
Total	15	.262	1.029	3.629	3.467	2.867	1.396
Contrasts							
Sources	1	.125	.563	3.063	9.000[c]	4.000[d]	3.063
Levels	1	.063	.563	.562	0.000	1.000	.063
Source x level	1	.113	.100	4.225	10.000[e]	4.900[c]	3.600

[a]P < .041.
[b]P < .006.
[c]P < .06.
[d]P < .08.
[e]P < .05.

Table A.5. Analysis of variance of ADG Paper 2, Experiment 1

		Mean Squares			
Source	df	ADG wk 1	ADG, wk 2	ADG, wk 3	ADG, wk 4[a]
Block	11	.134	.083	.245	.468[b]
Treatment	2	.230	.370[c]	.282	.504
Error	22	.073	.046	.132	.180
Total	35	.101	.076	.176	.292
Contrasts					
Basal vs supplemented	1	.442[b]	.737[d]	.534	.798[e]
Sources	1	.0172	.003	.031	.174

[a] df = 34.

[b] P < .03.

[c] P < .002.

[d] P < .0006.

[e] P < .05.

Table A.6. Analysis of variance of ADFI. Paper 2, Experiment 1

		Mean Squares			
Source	df	ADFI, wk 1	ADFI, wk 2	ADFI, wk 3	ADFI, wk 4[a]
Block	11	.073	.120[b]	.382	.405
Treatment	2	.201	.388[c]	.311	.870
Error	22	.067	.050	.260	.331
Total	35	.076	.091	.301	.386
Contrasts					
Basal vs					
supplemented	1	.344[d]	.749[e]	.621	1.630[b]
Sources	1	.057	.028	.002	.008

[a] df = 34.

[b] P < .04.

[c] P < .003.

[d] P < .033.

[e] P < .0008.

Table A.7. Analysis of variance of Gain to Feed (G/F). Paper 2, Experiment 1

		Mean Squares			
Source	df	G/F, wk 1	G/F, wk 2	G/F, wk 3	G/F, wk 4[a]
Block	11	.141	.034	.082	.211[c]
Treatment	2	.223	.062[b]	.034	.055
Error	22	.129	.016	.089	.066
Total	35	.138	.024	.084	.113
Contrasts					
Basal vs					
supplemented	1	.445	.124[c]	.066	.021
Sources	1	.001	.001	.002	.085

[a] df = 34.

[b] P < .04.

[c] P < .02.

Table A.8. Analysis of variance of antibody response to KLH injection. Paper 2, Experiment 1

		Mean Squares			
Source	df	Primary IgM[a]	Secondary IgM[b]	Secondary IgG[b]	Primary IgM[c]
Block	5	.005	.002	.090	.009
Treatment	2	.010	.005	.074	.009
Error	10	.003	.007	.041	.003
Total	17	.005	.005	.057	.005
Contrasts					
Basal vs supplemented	1	.015	.007	.045	.015
Sources	1	.004	.001	.142	.002

[a]Injected pigs with KLH after 1 wk of dietary treatment. Response measured 2 wk after injection.

[b]Injected KLH a second time after 3 wk of dietary treatment. Response measured 1 wk following injection. df = 16.

[c]Injected KLH after 3 weeks of dietary treatment. Response measured 1 wk following injection.

Table A.9. Analysis of variance of total leukocyte count and alkaline phosphatase activity. Paper 2, Experiment 1

		Mean Squares			
Source	df	Pretreat WBC[a]	Week 2 WBC[a]	Week 4 WBC[a]	ALK, IU
Block	5	19.85	90.05	15.02	9,423
Treatment	2	1.60	11.52	24.34	32,689
Error	10	8.80	32.15	18.18	15,411
Total	17	11.18	46.75	17.97	15,703
Contrasts					
Basal vs supplemented	1	1.02	5.52	46.24	40,797
Sources	1	2.18	17.52	2.43	24,580

[a]Units = millions per mL.

Table A.10. Analysis of variance of pretreatment proliferation to mitogens. Paper 2, Experiment 1[a]

		Mean Squares			
Source	df	RPMI, none	ConA, 10	PHA, 5	PHA, 1
Block	5	43	62,019,729	564,551,419[b]	1,626,408,607
Treatment	2	2	160,587,432	4,664,083,510	78,007,475
Error	9	.9	122,612,925	1,159,565,516	586,622,658
Total	16	35.2	108,424,364	299,948,523	84,797,887
Contrasts					
Basal vs supplemented	1	.8	24,277,035	465,682,932	91,701
Sources	1	.5	266,120,948	10,814,294,956[b]	4,837,114

[a] Mitogen units = μg/mL.
[b] $P < .02$.
[c] $P < .01$.

Table A.10 continued

		Mean Squares	
Source	df	PWM, .1	PWM, .01
Block	5	1,174,284,905[c]	43,555,373
Treatment	2	375,625,893	23,250,313
Error	9	190,595,906	18,684,758
Total	16	521,127,466	27,027,520
Contrasts			
Basal vs supplemented	1	11,545,961	9,264,618
Sources	1	361,225,413	6,705,698

Table A.11. Analysis of variance of proliferation to mitogens following two weeks of treatment. Paper 2, Experiment 1[a]

		Mean Squares			
Source	df	RPMI, none	ConA, 10	ConA, 5	PHA, 5
Block	5	285,270	3,538,212,828[b]	849,269,755[c]	4,261,926,763[d]
Treatment	2	25,468	4,377,989,621[e]	2,081,826,192[b]	531,362,171
Error	10	280,794	318,312,088	254,095,410	524,957,400
Total	17	252,068	174,294,748	64,417,384	162,481,954
Contrasts					
Basal vs supplemented	1	5,980	5,984,775,895[e]	3,034,669,381[f]	666,491,672
Sources	1	44,896	2,771,203,347[g]	1,128,983,002	396,232,669

[a] Mitogen units = μg/mL.

[b] $P < .0008$.

[c] $P < .05$.

[d] $P < .03$.

[e] $P < .002$.

[f] $P < .006$.

[g] $P < .02$.

[h] $P < .0001$.

[i] $P < .003$.

[j] $P < .0006$.

[k] $P < .004$.

Table A.11 continued

		Mean Squares		
Source	df	PHA, 1	PWM, .1	PWM, .01
Block	5	7,965,522,536[h]	848,680,346[i]	62,411,995[j]
Treatment	2	2,651,987,480[d]	578,963,470[d]	52,563,802[k]
Error	10	449,131,325	103,880,929	5,247,123
Total	17	291,899,417	378,831,645	27,626,989
Contrasts				
Basal vs				
supplemented	1	1,790,573,330	800,635,320[g]	97,696,751[e]
Sources	1	3,513,401,630[g]	357,291,620	7,430,854

Table A.12. Analysis of variance of proliferation to mitogens following four weeks of treatment. Paper 2, Experiment 1[a]

		Mean Squares			
Source	df	RPMI, none	ConA, 10	ConA, 5	PHA, 5
Block	5	3,787,853	2,258,128,657[b]	20,491,039	23,224,521,500
Treatment	2	5,726,104[b]	1,151,279,694	6,036,984	15,345,543,237
Error	10	1,394,402	628,772,750	7,259,662	26,412,162,069
Total	17	2,607,970	116,946,648	11,007,399	24,172,665,570
Contrasts					
Basal vs supplemented	1	557,511	539,269,025	11,329,956	12,298,181,575
Sources	1	10,894,696[c]	1,763,290,364	744,012	18,392,904,901

[a] Mitogen units = μg/mL.

[b] $P < .05$.

[c] $P < .02$.

[d] $P < .03$.

[e] $P < .04$.

Table A.12 continued

		Mean Squares		
Source	df	PHA, 1	PWM, .1	PWM, .01
Block	5	8,115,877,862[c]	756,183,142[d]	43,537,504[e]
Treatment	2	411,689,138	28,900,467	6,957,588
Error	10	1,512,771,885	173,475,810	11,595,883
Total	17	3,325,327,731	327,851,455	20,444,796
Contrasts				
Basal vs supplemented	1	2,845,407	2,059,703	13,802,463
Sources	1	820,532,870	55,741,231	112,714

Table A.13. Analysis of variance of pretreatment lymphocyte cell surface markers. Paper 2, Experiment 1

		Mean Squares			
Source	df	MHC classII	CD2	CD4	CD8[a]
Block	5	43.71	118.06	52.22[b]	46.34
Treatment	2	1.37	82.45	11.24	14.58
Error	8	25.21	82.31	13.08	37.37
Total	15	28.20	94.27	25.88	37.32
Contrasts					
Basal vs supplemented	1	.49	82.35	39.46	1.11
Sources	1	12.71	32.76	6.03	11.28

[a] df = 16.

[b] $P < .05$.

[c] df = 12.

Table A.13 continued

		Mean Squares	
Source	df	MAC Øc	RATIO
Block	5	287.00	.13
Treatment	2	60.05	.02
Error	8	125.92	.43
Total	15	17.08	.07
Contrasts			
Basal vs supplemented	1	9.63	.05
Sources	1	110.07	.01

Table A.14. Analysis of variance of lymphocyte cell surface markers after 2 wk of dietary treatment. Paper 2, Experiment 1

		Mean Squares			
Source	df	MHC classII	CD2	CD4[a]	CD8
Block	5	90.92	102.51	45.96	110.57
Treatment	2	24.62	15.95	42.51	27.97
Error	8	75.28	73.84	39.24	55.11
Total	15	73.73	75.67	42.11	69.98
Contrasts					
Basal vs supplemented	1	57.60	6.71	78.57	24.61
Sources	1	5.26	19.39	5.83	44.26

[a] df = 14.

[b] df = 12.

Table A.14 continued

		Mean Squares	
Source	df	MAC Ø[b]	RATIO
Block	5	203.25	.029
Treatment	2	100.09	.047
Error	7	148.01	.026
Total	14	163.04	.030
Contrasts			
Basal vs supplemented	1	120.72	.13
Sources	1	.487	.011

Table A.15. Analysis of variance of lymphocyte cell surface markers after 4 wk of dietary treatment. Paper 2, Experiment 1

		Mean Squares			
Source	df	MHC classII	CD2	CD4	CD8
Block	5	146.60	194.43	98.32	180.36
Treatment	2	478.31	254.98	35.78	233.95
Error	10	235.16	62.48	126.49	60.44
Total	17	237.71	123.94	107.53	116.12
Contrasts					
Basal vs supplemented	1	940.24	473.06[a]	24.32	463.47[a]
Sources	1	16.38	36.89	47.24	4.43

[a] $P < .02$.

[b] $P < .04$.

Table A.15 continued

		Mean Squares	
Source	df	MAC Ø	RATIO
Block	5	163.58	.17
Treatment	2	356.37[b]	.07
Error	10	76.92	.08
Total	17	135.28	.11
Contrasts			
Basal vs			
supplemented	1	685.83[a]	.102
Sources	1	26.91	.04

Table A.16. Analysis of variance of gain, ADFI, thymus weight and antibody response to *Serpulina hyodysenteriae* infection. Paper 2, Experiment 2

		Mean Squares				
Source	df	Gain, kg	ADFI, kg	Thymus, g	Thymus, g/kg bw	Antibody, OD
Block	1	.201	.240	496.86	.647	.0001
Treatment	2	16.602	.127[a]	2,315.85	.679	.0006
Error	2	18.472	.000	185.54	.169	.0016
Total	5	14.076	.099	636.76	.469	.0009
Contrasts						
Basal vs supplemented		10.641	.213[a]	651.21	.456	.0009
Sources		22.563	.040[a]	1,664.64	.903	.0003

[a] $P < .0001$.

Table A.17. Analysis of variance of clinical signs of swine dysentery for first five days after challenge. Paper 2, Experiment 2

		Mean Squares				
Source	df	Clinical score 1d	Clinical score 2d	Clinical score 3d	Clinical score 4d	Clinical score 5d
Block	1	.882	.060	.540	2.535[a]	2.535[a]
Treatment	2	.207	.047	.855	.185	.105
Error	2	.647	.560	.605	.035	.015
Total	5	.518	.255	.692	.595	.555
Contrasts						
Basal vs supplemented	1	.403	.003	.608	.368[b]	.188[c]
Sources	1	.010	.090	1.103	.003	.023

[a]P < .01.

[b]P < .08.

[a]P < .07.

Table A.18. Analysis of variance of ADFI, ADG, and feed efficiency. Paper 3

		Mean Squares		
Source	df	ADFI, kg	ADG, kg	Gain/feed
Block	5			
Sex	1	.0005	.0002	.0001
Block(sex)	4	.0662	.0140	.0058
Treatment	2	.0775	.0511	.0187
Trt x sex	2	.0139	.0030	.0000
Error	8	.0394	.0167	.0042
Total	17	.0449	.0176	.0055
Contrasts				
Basal vs supplemented	1	.1405	.1012[a]	.0366
Sources	1	.0145	.0011	.0008[b]

[a]P < .04.

[b]P < .02.

Table A.19. Analysis of variance of organ size. Paper 3

		Mean Squares		
Source	df	Thymus g/kg bw	Spleen, g/kg bw	Liver g/kg bw
Block	5			
Sex	1	.164	.41	6.919
Block(sex)	4	.021	.61	27.78
Treatment	2	.613	.18	2.330
Trt x sex	2	.049	.50	11.050
Error	8	.526	.14	10.443
Total	17	.340	.31	13.437
Contrasts				
Basal vs supplemented	1	1.134	.16	3.010
Sources	1	.092	.19	1.650

Table A.20. Analysis of variance of blood chemistry parameters. Paper 3

		Mean Squares			
Source	df	Creatinine, mg/dL	CPK, U/L	Amylase, IU/L	BUN/creat ratio
Block	5				
Sex	1	.0022	41,761	547,058	7.3472
Block(sex)	4	.1022	32,733	398,970	33.0572
Treatment	2	.0339	103,029	153,523	.5505
Trt x sex	2	.0039	18,132	87,868	18.7406
Error	8	.0156	63,811	215,573	5.3322
Total	17	.0359	54,441	255,900	12.9892
Contrasts					
Basal vs supplemented	1	.0469	178,506	272,484	.0803
Sources	1	.0208	27,552	34,561	1.0208

[a]Abbreviations used: CPK = creatinine phosphokinase; BUN = blood urea nitrogen; A/G = albumin/globulin; ALT = alanine amino transferase; gamma GT = gamma globulin; ALK = alkaline phosphatase; LDH = lactate dehydrogenase; AST = aspartate aminotransferase.

[b]Basal differs from supplemented $P < .004$.

[c]Basal differs from supplemented $P < .034$.

[d]Basal differs from supplemented $P < .0001$.

[e]Basal differs from supplemented $P < .01$.

Table A.20 continued

Source	df	Mean Squares			
		Osmolality	A/G ratio	Glucose, mg/dL	Cholesterol, mg/dL
Block	5				
Sex	1	3.7355	.0450	112.50	501.39
Block(sex)	4	13.6611	.0578	267.33	673.55
Treatment	2	10.5350	.0022	436.17	147.06
Trt x sex	2	6.8439	.0067	36.17	698.72
Error	8	7.6578	.0694	52.25	274.39
Total	17	9.0824	.0500	149.64	413.41
Contrasts					
Basal vs supplemented	1	3.0625	.0044	870.25[b]	23.36
Sources	1	18.0075	.0000	2.08	270.75

Table A.20 continued

Source	df	Mean Squares			
		ALT, IU/L	Gamma GT, IU/L	ALK, IU/L	Na, mmol/L
Block	5				
Sex	1	8.00	80.22	5,338.89	.8888
Block(sex)	4	4.44	126.61	3,279.11	.2222
Treatment	2	126.39	8.17	148,920.50	3.5555
Trt x sex	2	15.17	1.39	3,976.05	1.5556
Error	8	30.86	36.28	2,458.44	3.2222
Total	17	32.70	52.71	20,230.35	2.2222
Contrasts					
Basal vs supplemented	1	200.69[c]	16.00	29,750.25[d]	1.7778
Sources	1	52.08	.33	270.75	5.3333

Table A.20 continued

		Mean Squares			
Source	df	K, mmol/L	Cl, mmol/L	Ca, mg/L	P mg/L
Block	5				
Sex	1	.0356	464.11	.3472	.0272
Block(sex)	4	.3156	605.16	.3222	2.5056
Treatment	2	1.5739	677.29	.0689	3.0339
Trt x sex	2	.2239	529.71	.3289	1.2006
Error	8	.2431	637.80	.1414	1.9506
Total	17	.4022	611.82	.2096	2.0070
Contrasts					
Basal vs supplemented	1	2.6678[e]	320.41	.1344	5.3669
Sources	1	.4800	1,034.16	.0033	.7008

Table A.20 continued

Source	df	Mean Squares: BUN, mg/dL	Total protein, g/dL	Albumin, g/dL	LDH, IU/L	AST, IU/L
Block	5					
Sex	1	4.5000	.5339	.1605	1,012.50	53.39
Block(sex)	4	36.3333	.6144	.1078	1,190.44	240.22
Treatment	2	3.1667	1.0617	.1800	1,804.06	705.06
Trt x sex	2	21.5000	.0506	.0156	740.17	246.06
Error	8	5.1667	.2561	.0528	1,625.86	126.05
Total	17	2.4313	.4274	.0829	1,404.09	230.89
Contrasts						
Basal vs supplemented	1	6.2500	2.1025[c]	.3600[c]	3,560.11	1,308.02
Sources	1	.0833	.0208	.0000	48.00	102.08

Table A.21. Analysis of variance of hematologic parameters. Paper 3

		Mean Squares			
Source	df	RBC	HGB	HCT	MCV
Block	5				
Sex	1	.0006	6.361	58.68	65.74
Block(sex)	4	1.497	1.846	28.03	26.08
Treatment	2	1.017	.403	34.30	.751
Trt x sex	2	.651	1.077	40.32	5.67
Error	8	.390	.289	10.39	15.25
Total	17	.732	1.118	23.72	17.94
Contrasts					
Basal vs supplemented	1	1.734	.667	56.00[b]	.54
Sources	1	.301	.141	12.61	.96

[a]Abbreviations used: RBC = red blood cell; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; WBC = white blood cell; SEG = segmented neutrophils ; BAND = band neutrophils; LYMPH = lymphocytes; MONO = monocytes; EOSIN = eosinophils.

[b]Basal differs from supplemented $P < .05$.

[c]Basal differs from supplemented $P < .03$.

Table A.21 continued

Source	df	Mean Squares MCH	MCHC	WBC	SEG
Block	5				
Sex	1	7.347	1.076	13.18	18.00
Block(sex)	4	.646	1.056	50.57	51.33
Treatment	2	1.117	2.967	19.59	180.50
Trt x sex	2	.267	3.057	2.50	19.50
Error	8	1.289	1.080	17.74	78.83
Total	17	1.353	1.529	23.59	73.76
Contrasts					
Basal vs supplemented	1	2.151	4.914	37.01	342.25
Sources	1	.083	1.021	2.17	18.75

Table A.21 continued

		Mean Squares			
Source	df	BAND	LYMPH	MONO	EOSIN
Block	5				
Sex	1	.0556	24.50	2.722	.500
Block(sex)	4	.2778	42.11	2.222	5.778
Treatment	2	.5000	239.06	2.389	.222
Trt x sex	2	.0556	24.50	1.056	.667
Error	8	.2778	65.78	.8056	2.361
Total	17	.2647	73.29	1.471	2.589
Contrasts					
Basal vs supplemented	1	1.0000	448.03[c]	1.778	.1111
Sources	1	.0000	30.08	3.000	.3333

Table A.22. Analysis of variance of proliferative response of peripheral blood cells to mitogens and *S. hyodysenteriae* antigen. Paper 3

		Mean Squares				
Source	df	PHA, 5	PHA, 2.5	PHA, 12.5	PWM, .001	PWM, .01
Block	5					
Sex	1	1,532.4	25,462.7	19,987	2.156	32.29
Block(sex)	4	4,207.6	9,499.2	10,150	.852	445.4
Treatment	2	793.8	63.2	3,381	1.466	954.8
Trt x sex	2	3,592.9	6,017.1	4,059	1.601	662.0
Error	8	3,017.0	2,413.0	3,419	1.384	160.4
Total	17	3,016.0	5,583.7	6,048	1.353	372.4
Contrasts						
Basal vs supplemented	1	1436.3	92.10	6,096	.601	1,684[a]
Sources	1	151.4	34.21	665.7	2.33	225.0

[a] $P < .01$.

Table A.22 continued

		Mean Squares			
Source	df	PWM, .1	S. HYO, 1	S. HYO,10	S. HYO, 25
Block	5				
Sex	1	5882	16.89	.071	10.38
Block(sex)	4	5210	124.47	345.6	617.1
Treatment	2	2745	47.18	58.18	106.7
Trt x sex	2	13330	4.48	268.9	470.0
Error	8	5491	79.17	241.7	353.0
Total	17	6047	73.59	233.4	379.8
Contrasts					
Basal vs					
supplemented	1	3675	35.26	97.19	205.6
Sources	1	1814	59.09	19.18	7.825

Table A.23. Analysis of variance for porcine antibody response following vaccination with *S. hyodysenteriae*. Paper 3

		Mean Square					
Source	df	14 d[a]	28 d[a]	38 d[a]	44 d[a]	38 d[b]	44 d[b]
Block	5						
Sex	1	.0003	.0174	.0000	.0339	.0061	.0002
Block(sex)	4	.0007	.0106	.0597	.0851	.0179	.0211
Treatment	2	.0018	.0202	.0588	.0404	.0357	.0438
Trt x sex	2	.0004	.0288	.0158	.0146	.0046	.0210
Error	8	.0005	.0116	.0136	.0176	.0100	.0084
Total	17	.0006	.0147	.0294	.0364	.0147	.0170
Contrasts							
Basal vs supplemented	1	.0032[d]	.0040	.1131[c]	.0746	.0645[d]	.0872[c]
Sources	1	.0032	.0365	.0044	.0061	.0034	.0003

[a] heavy and light chain specific.
[b] gamma chain specific.
[c] $P < .02$.
[d] $P < .04$.

Table A.24. Analysis of variance for serum zinc, bone zinc, and bone ash. Paper 3

		Mean Square					
Source	df	14 d[a]	28 d[a]	38 d[a]	44 d[a]	44 d[b]	44 d[c]
Block	5						
Sex	1	.0014	.0481	.0156	.0032	27.88	1.18
Block(sex)	4	.0098	.0056	.0274	.0153	5.93	911.56
Treatment	2	.1606	.2927	.1106	.1603	88.18	23,292
Trt x sex	2	.0124	.0283	.0097	.0067	10.14	1,178.36
Error	8	.0099	.0071	.0217	.0032	8.05	637.21
Total	17	.0276	.0452	.0318	.0247	18.39	3,393.26
Contrasts							
Basal vs supplemented	1	.3173[d]	.5852[e]	.2147[f]	.3007[e]	173.36[f]	42,855.9[e]
Sources	1	.0040	.0010	.0065	.0200[g]	3.00	3,727.69[h]

[a]Serum zinc, ppm.

[b]Bone Ash, %.

[c]Bone zinc, ppm.

[d]$P < .0005$.

[e]$P < .0001$.

[f]$P < .02$.

[g]$P < .04$.

[h]$P < .05$.

Table A.25. Analysis of variance of feed intake for C3H/HeN mice. Paper 4

Source	df	Mean Squares	
		Intake, prechallenge	Intake, post challenge
Treatment	1	9.924[a]	.044
Error	5	.595	3.069
Total	6	2.15	2.56

[a]$P < .05$.

Table A.26. Analysis of variance for cecal weight and organisms per gram ceca three days following infection. Paper 5

Source	df	Mean Squares	
		Cecal weight	Organisms x 10^4 per g ceca
Treatment	4	.007	3,290
Error	10	.011	5,329
Total	14	.01	66,446

Table A.27. Analysis of variance for cecal lesions 10 d following infection. Paper 5

Source	df	Mean Squares
Treatment	4	1.8
Error	10	1.4
Total	14	1.5

Table A. 28. Analysis of variance for histopathologic analysis of cecal lesions 3 d following infection. Paper 5

		Mean Square				
Source	df	Gland H x W	Mitotic figures	Epithelial damage	Lamina propria	Total score
Treatment	4	1.21[a]	9.30	.55	1.54[b]	17.69
Error	9	.22	5.83	.13	.37	5.19
Total	13	.527	6.90	.13	.73	9.03

[a]P < .02.
[b]P < .04.

Table A. 29. Analysis of variance for histopathologic analysis of cecal lesions 10 d following infection. Paper 5

		Mean Square				
Source	df	Gland H x W	Mitotic figures	Epithelial damage	Lamina propria	Total score
Treatment	4	.10	17.77	1.57	3.43[a]	52.93[a]
Error	10	.53	5.93	.87	.8	11.67
Total	14	.41	9.31	1.07	1.55	23.46

[a]P < .03.